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President of the Association of Applied Biologists, 1946–48

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THE SIGNIFICANCE OF PLANT DISEASES IN GREAT BRITAIN

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ADDRESS OF THE PRESIDENT OF THE ASSOCIATION OF APPLIED BIOLOGISTS, DELIVERED TO THE ANNUAL MEETING ON FRIDAY, 18 FEBRUARY 1949

It was Disraeli (1845) who once wrote 'to be conscious that you are ignorant is a great step to knowledge' and I find that a comforting thought in trying to grapple with a subject about which we still are largely ignorant. I know that figures have frequently been quoted in text-books and elsewhere to show the monetary loss caused by this or that disease, but these figures have rarely been based on firm foundations and usually little or no account has been taken of the other interacting factors involved. Indeed, I have seen income and expenditure accounts that have been drawn up showing the normal value of crop yield in the absence of, say, seed treatment; the cost of treatment against a particular disease; and the net gain derived from applying the treatment. But where this was done for several diseases on the same crop, simple arithmetic by the sceptic showed that the total net gain sometimes far exceeded the normal total value of the crop! If anyone is expecting to hear how much apple scab or potato blight or some other disease is costing us annually he will be disappointed. Nor do I intend to give many lists of diseases in order of their economic importance: though, if not instructive, it might be amusing to compare such lists prepared by a couple of dozen of knowledgeable plant pathologists with widely different interests. My intention is to discuss the chief methods by which more or less accurate knowledge of diseases is being slowly acquired in Britain, and to give a few examples of results already obtained, in the hope that further interest may be aroused in the accurate assessment of damage by plant diseases.

There are three main ways in which the significance of plant diseases can be studied: by intelligence work involving the slow accumulation of data about all kinds of diseases over many years; by extensive surveys of single diseases or groups of diseases on a national or regional basis; and by intensive research methods carried out on plot and field scale. Each method has its advocates and each its critics, but the plain fact is that all are essential, and each is complementary to the others. It is comparatively easy to underrate or overrate their value, though disparagement mostly means merely that too much is being expected of the method. In general, it is a waste of time to try and extract quantitative results from routine intelligence work, and it is equally dangerous to accept for the country as a whole, what has been shown to be true for a particular district, or even for a particular plot. May I then look a little more closely at these three methods?

The groundwork of knowledge about plant diseases in Britain was laid by Berkeley, Cooke, Massee and Salmon, but it was not until 1917 that intelligence work on plant disease was properly organized. In that year the Technical Committee of the War-Time Food Production Department set up a Sub-Committee to advise the Department on questions relating to pests and diseases. Among other things, the Sub-Committee inaugurated a plant-disease survey of England and Wales by means of a system of monthly reports prepared by specially qualified honorary correspondents in all parts of the country. This intelligence work has persisted without a break until now, and altogether about 200 collaborators have taken part in it, though for most of the time the bulk of the reporting has been done by Provincial Advisory Mycologists and their staffs. Thanks largely to the thorough and meticulous way in which the late Dr G. H. Pethybridge organized the work between 1923 and 1936, the wealth of information accumulated through this Intelligence Service is second to none in the world. The bare bones of it have been published in a series of nine Reports issued by the Ministry of Agriculture and Fisheries as Miscellaneous Publications or Bulletins (Min. Agric. 1918–48), but the original reports have been carefully preserved and all the information is available to those who appreciate that it can serve a purpose in throwing light on some aspect or other of many different problems.

I would like to refer briefly to some of the things this Intelligence Service tells us, and here I must draw mainly on experience in England and Wales, for in Scotland organized intelligence work on any scale is quite a recent development, though already giving much very welcome information. In the first place it gives an overall and general picture of the relative importance of different diseases. Fortunately, in Britain we do not suffer the devastating losses that sometimes occur in countries where single crops cover large areas in one district, but the total loss from disease, if it could be measured, would surely be staggering. About 1000 different diseases have been recorded in the series of reports referred to, and that is a conservative estimate, for as a general rule fungi or viruses that cause disease in a wide range of hosts have been included only once. From this large number I thought it might be helpful if I selected the forty or so diseases or groups of diseases which in my judgement are the most important economic ones occurring in Great Britain at the present time. They are:

Mildew (*Erysiphe graminis* DC.), foot rots (*Ophiobolus graminis* (Sacc.) Sacc., *Cercosporella herpotrichoides* Fron and *Fusarium* spp.), and yellow rust (*Puccinia glumarum* (Schm.) Erikss. & Henn.) of cereals, with a certain reservation to be referred to later.

Blight (*Phytophthora infestans* (Mont.) de Bary), dry rot (*Fusarium* spp.), and virus diseases of potato.

Virus yellows, and downy mildew (*Peronospora schachtii* Fuckel) of sugar beet.

Chocolate spot (*Botrytis cinerea* Fr. and *B. fabae* Sardiña) of bean.

Leaf and pod spot, and foot rot (*Ascochyta* spp.) of pea.

Clover rot (*Sclerotinia trifoliorum* Erikss.).

Club root (*Plasmodiophora brassicae* Woron.), and virus diseases of crucifers.

Bacterial soft rot (*Bacterium carotovorum* (Lehm.) L. R. Jones), and associated diseases of vegetable crops.

Mosaic, and grey mould (*Botrytis cinerea* Fr.) of lettuce.

White rot (*Sclerotium cepivorum* Berk.), Botrytis rots (*Botrytis allii* Munn and *B. byssoidea* J. C. Walker), and downy mildew (*Peronospora destructor* (Berk.) Casp.) of onion.

Stem and fruit rot (*Didymella lycopersici* Kleb.), leaf mould (*Cladosporium fulvum* Cooke), and root rots of tomato.

Brown rot (*Sclerotinia fructigena* Aderh. & Ruhl.), scab (*Venturia inaequalis* (Cooke) Wint. and *V. pirina* Aderh.), and canker (*Nectria galligena* Bres.) of apple and pear.

Bacterial canker (*Pseudomonas mors-prunorum* Wormald), and silver leaf (*Stereum purpureum* (Fr.) Fr.) of plum and cherry.

American gooseberry mildew (*Sphaerotheca mors-uvae* (Schw.) Berk.).

Red core (*Phytophthora fragariae* Hickman), and virus diseases of strawberry.

Virus diseases of raspberry.

Nettlehead, and verticillium wilt (*Verticillium albo-atrum* Reinke & Berth. and *V. dahliae* Kleb.) of hop

And, among ornamental plants, carnation wilt (*V. cinerescens* Wollenw.), corm rots (especially *Botrytis gladiolorum* Timmerm.) of gladiolus, virus diseases of narcissus and iris, black spot (*Actinonema rosae* (Lib.) Fr.) of rose, and fire (*Botrytis tulipae* Lind) of tulip. To these I would add the effects of cucumber mosaic virus in many hosts.

It would take too long to give all the reasons for my choice, but I should perhaps explain that in compiling the list I had an eye on the monetary value of the crop as well as its contribution to the national larder, and on the extent to which potentially destructive diseases are or are not adequately controlled. Many of them are diseases for which there is as yet no satisfactory control measure. For comparative purposes it may be as well to bear in mind that the annual pre-war value of the potato crop in Great Britain amounted to about £14 million, or nearly twice the value of the fruit crop and three times that of wheat, barley or sugar beet. The annual retail value of the flower crop was £15 million, and four crops—bulbs, carnations, chrysanthemums and roses—accounted for two-thirds of it. I deliberately omitted deficiency diseases because I find it very difficult to assess their real and relative value, and because I feel that to some extent their significance is a transient one bound up with war-time difficulties in the supply of fertilizers and choice of land.

To one accustomed to handling plant-disease records it is not difficult to detect regional differences in the prevalence and severity of some diseases. Thus, Scottish oat crops are usually free from crown rust (*Puccinia coronata* Corda); powdery scab (*Spongospora subterranea* (Wallr.) Lagerh.) of potato is of little significance in the east and south-east of England and Wales, while often troublesome in the wetter districts

of the west and north; *Rosellinia necatrix* Prill., the cause of white root rot of many different plants, has been found only very occasionally outside the south and south-west; marsh spot (manganese deficiency) of peas is troublesome only in low-lying marshlands near the sea, such as Romney Marsh in Kent, Foulness Island in Essex, and some parts of Lincolnshire; ring spot (*Mycosphaerella brassicicola* (Duby) Oudem.) of brassicas, though widely distributed, is important only in the south-west and Wales; antirrhinum rust (*Puccinia antirrhini* Diet. & Holw.) has not secured the grip in Scotland and northern England that it has in the south; and the leaf diseases of narcissus, that have been so damaging in the Isles of Scilly and western Cornwall, are insignificant in most other districts. On the other hand, it is not possible to extract quantitative data apart from quite general statements, such as that loose smut (*Ustilago tritici* (Pers.) Rostr.) of wheat rarely affects more than 1% of the ears except in a very susceptible variety like Vilmorin; or that, in general, losses from black leg (*Bacterium phytophthorum* (Appel) Burgwitz) of potatoes do not exceed 2% in normal seasons or 10% in seasons specially favourable to it.

This leads me on to what I regard as one of the most important contributions the intelligence reports are able to make. They permit the commoner diseases to be considered in proper perspective, and they provide a basis for sound judgement when epidemics of disease cause alarm. Two illustrations of this must suffice. The exceptionally severe epidemic of take-all (*Ophiobolus graminis* (Sacc.) Sacc.) in cereals in 1948 (Moore, 1948*b*) gave rise to much concern on all sides. The records show that in pre-war days when rotations were normal, about one year in five was a bad take-all year, particularly in areas overlying chalk and limestone formations. They also show that the more continuous cropping with cereals since 1939 so increased the risks from take-all that in 1943–8 every other year was a take-all year. Moreover, they indicated that in 1948, when the disease was more widespread and destructive in England than at any other time since it was first recognized here some thirty-five years ago, severe attacks occurred well outside the usual danger areas, and on land where wheat and barley had not been grown the previous year. Fortunately, it was possible to interpret these observations in the light of S. D. Garrett's (1936–48) researches on take-all at Rothamsted, and so to reassure farmers to some extent about the future.

A similar position arose during and after the devastating epidemic of chocolate spot (*Botrytis cinerea* Fr. and *B. fabae* Sardiña) of field and broad beans in 1944 (Moore, 1944). Here is a disease that is responsible for appreciable losses in the average season, while in years like 1935 and 1944, when severe epidemics occurred over the greater part of the country, yields were commonly *said* to be reduced by 50%, and certainly some crops were rendered useless and had to be ploughed in. Yet the intelligence reports since 1917 clearly indicate that general epidemics of chocolate spot occur only about one year in six; that, in addition, there are local epidemics in the south and west about as infrequently; and that every one year in four the disease can scarcely be found. The conclusions drawn from these records in

1944, when concern was being expressed about the future of the crop, have been amply justified, for there has been no further general epidemic of chocolate spot, and it was sub-normal in three of the four seasons that have since elapsed. The indications are that chocolate spot becomes epidemic only when severe late spring frosts are followed by warm, wet weather in June or July, but epidemiological research is required before the accumulated observations on the disease can be used to the best advantage.

If the records help sometimes to allay alarm, however, they can also be used to foresee possible trouble ahead. Since 1940 brown rot (*Sclerotinia fructigena* Aderh. & Ruhl.) of apples and pears has already become a real bugbear, and almost every year, instead of one in at most three or four, is a 'brown rot year'. Canker (*Phoma lingam* (Fr.) Desm.) of broccoli and other brassicae, hitherto of importance only in northern districts, has begun to make its presence felt farther south, and the routine hot water treatment to control it, recently introduced as a provincial service in Yorkshire and elsewhere, is a timely precaution. Another disease that bids fair to become a menace in the future is grey bulb rot (*Sclerotium tuliparum* Kleb.) of tulip. It was unknown in Britain before 1922 and five years later only six attacks had been observed. By 1934 thirty-eight outbreaks were known, and in the next five years forty-four more were added. The numbers themselves are unimportant—for there are probably many others unrecorded—but it is significant that this disease is now widely distributed throughout Britain. Once the soil is contaminated with the sclerotia of the parasite it remains contaminated for several years, and tulips planted in it are usually killed before they come above ground. Unless efforts are made to control it, and that is not an easy matter, this disease in my opinion will soon assume far greater significance than fire (*Botrytis tulipae* Lind). I also venture to think that gangrene (*Phoma foveata* Foister) of potatoes, leaf blotch (*Heterosporium allii* Ell. & Mart. var. *cepivorum* Nich. & Aggéry) of onion, Alternaria blight (*Alternaria solani* (Ell. & Mart.) Sor.) of tomato, and ink disease (*Mystrosporium adustum* Massee) of iris may well become far more troublesome in the future than they have been in the past.

Other illuminating results of studying the information available are the discovery that epidemics of certain diseases may occur in waves at long intervals, and that changes in cultivation and cropping, as well as the application of the results of research, may have a profound influence on the significance of specific diseases. There have been two epidemic phases of stem rot (*Didymella lycopersici* Kleb.) of tomato in this country. It has been known here since 1885, and between 1906 and 1909 was very destructive in the Lea Valley. Subsequently it decreased in virulence and until 1938, though still about, was of no consequence. The following year it began to flare up again, and by 1945 had reached a new peak of destructiveness over most of England and Wales. No really effective means has been found of dealing with it, yet since 1946 the disease seems to have been decidedly on the wane. Another example of this is asparagus rust (*Puccinia asparagi* DC.), known in England

since 1865. For thirty years it attracted no attention, and then suddenly in 1895 became epidemic in several parts of the country. It was also severe in the Evesham area in 1904–6, but subsequently remained quiescent until the really hot summer of 1933, when substantial attacks were seen in East Anglia and Worcestershire. It hung about for a year or two, but has been reported only once since 1935.

Twenty years ago wart disease (*Synchytrium endobioticum* (Schilb.) Perc.) of potato would certainly have figured in my list of principal diseases, but its spread was greatly reduced as a result of the operation of the Wart Disease of Potatoes Orders of 1923 and 1929, and now that some two-thirds of the potatoes grown in this country are immune varieties it is of no real economic import. Cucumber blotch (*Cercospora melonis* Cooke), which virtually wiped out the crop in North London at the beginning of the century, ceased to have any significance after the introduction of the variety Butcher's Disease Resister, which was derived from two unaffected plants in a crop otherwise destroyed by the disease at Dunstable. Again, for twenty years prior to 1930, stored daffodil bulbs constantly suffered very badly from basal rot (*Fusarium bulbigenum* Cooke & Massee). Research (Gregory, 1932; Hawker, 1935) showed that the disease could be avoided, and its spread prevented, by cool storage and by adding formalin or some other fungicide to the bath when the bulbs are given hot-water treatment against eelworm. Following the general adoption of these measures basal rot became so much a rarity that it was difficult to find sufficient affected bulbs for further research!

Other examples will doubtless occur to some of you. I will content myself with pointing out that the stripe diseases (*Helminthosporium* spp.) of cereals, so widespread and severe in the twenties and early thirties, are now of little consequence except on oats in Scotland and parts of western England, thanks largely to the adoption of seed disinfection with organo-mercury compounds; that hop downy mildew (*Pseudoperonospora humuli* (Miyabe & Tak.) G. W. Wilson), once of great concern to nearly all Europe, is now accepted here as almost a friendly resident which does not get out of hand except when both July and August are very wet; and that there has been a marked improvement in the health of potato stocks following the introduction in Scotland in 1931, and in England and Wales in 1940, of schemes for certifying growing crops in respect of their freedom from leaf roll and rugose mosaic—an improvement that can be obtained, and is slowly being obtained, with other crops subject to virus diseases. The framework of Health Certification Schemes is very largely dependent on the results of research, but once the schemes can be introduced, and the principles underlying them are generally understood and generally adopted, virus diseases should cease to occupy the very prominent position they now have in intelligence work.

If I have dwelt at length on the contribution of routine intelligence work in estimating the significance of plant diseases, it is because this side is a long way ahead of the other methods I referred to. But the Intelligence Service can be expected to give only qualitative results. These indicate the really important

economic diseases; they permit a proper perspective to be obtained of the prevalence and severity of these diseases in different seasons and different districts; they serve to draw attention to new and uncommon diseases; they enable one to judge the effects of the application of research to agriculture and horticulture; they can be used to direct attention to current problems; and they are always available to illuminate, supplement, and adjust the findings of special surveys and research.

A second method of studying the significance of plant diseases is by extensive surveys of single diseases, or groups of diseases, on a national or regional basis. Work along these lines is still in its infancy here, but a start has been made. The simplest form of it is when a special search is made for the presence or absence of a particular disease, the results of which may act as a corrective to routine intelligence information for any of the less common diseases. For many years onion smut (*Urocystis cepulae* Frost) was regarded as a very serious disease, the spread of which should be prevented at all costs. It was known to be prevalent in the south of Scotland, but in England it was thought, even as recently as 1941, to be restricted to small areas in the north and in Worcestershire, with a few isolated attacks elsewhere. During 1943 a special survey was made for its presence in onion-growing areas in England, and altogether 900 acres, or about one-twelfth of the total onion acreage at the time, were examined by Inspectors of the Ministry of Agriculture and Fisheries. The results indicated not only that the disease was quite generally distributed in the onion-growing parts of the Vale of Evesham and Bedfordshire, but that it is apparently much less harmful than was formerly supposed. Counts made in Bedfordshire revealed that although small plantings occasionally showed 30% infection, most plantings had only 0·5–2·5% of smutted seedlings, and these were distributed more or less uniformly over the fields. Similar presence or absence surveys are now being made to determine more accurately the distribution of red core (*Phytophthora fragariae* Hickman) of strawberries. The method is also useful in estimating the possible dangers of new diseases. The presence of bacterial canker of tomato was confirmed in England in 1942, and for the next three years special attention was paid to its occurrence. Altogether about 26 acres of outdoor tomatoes became known to be infected, or less than 1% of the average annual acreage in 1942–5, while under glass less than 0·1% of the average annual acreage during the same period was recognized as infected. Judged from the damage caused, the disease is not a potential source of trouble to the indoor crop, but has potentialities in the open, for the losses were substantial in a number of outbreaks, and in one instance more than one-quarter of 8000 plants were killed or severely damaged by it (Moore, 1947).

More elaborate surveys entail the use of simple and rapid but reliable methods of recording diseases quantitatively in the field. The Plant Pathology Committee of the British Mycological Society has recently devoted a good deal of attention to this subject (B.M.S. 1943–8) and has provided a lead for the Disease Assessment Committee recently set up by the Conference of Advisory Plant Pathologists, under the N.A.A.S., to promote further work on plant disease measurement. A valuable

by-product of the Society's work was the preparation of 'curves' (see Moore, 1948*a*) illustrating the progressive development of potato blight at a number of centres. These curves were based on records obtained while devising a method for estimating the amount of blight in a crop, and they clearly revealed that the course of blight differs widely in different parts of the country. There is, however, a lot of difficulty in creating the requisite amount of interest in this type of work, partly, I feel sure, because of confusion of thought between the methods essential for research and those more appropriate to extensive surveys. The two should be kept distinct and the simpler the survey method, provided it gives tangible and reliable results, the better. May I refer to an example of an extensive survey, carried out on simple lines, which yielded results of much value?

Onions became an important war-time crop. Acreages rose from 1800 to 12,000, and problems of pest and disease became matters of national importance. In 1943 a survey of onion diseases was carried out by Advisory Mycologists throughout England and Wales on a random selection of fields and storage houses. For virus diseases four or five random samples, of about 100 plants each, were examined across the diagonals of the field, and the percentage of affected plants calculated. For downy mildew and white rot three grades were used for recording fields. Grade I signified scattered plants slightly affected, Grade II small but definite patches in the crop where disease was moderately severe, and Grade III a general severe attack of downy mildew or extensive patches of white rot. Experience showed that some modifications in the grading were desirable and that such modifications need not lead to undue complexity. For stored bulbs, samples of twenty-five bulbs were drawn in four different places from the stock of each variety inspected, and the percentage infected with disease was recorded. About 500 crops, comprising 600 acres, or about 5% of the onion acreage, were inspected, and 550 tons of bulbs were sampled. The survey was repeated on a somewhat smaller scale in 1945. These surveys, which gave closely similar results in the two years, revealed that of the dozen or so diseases known to affect onions, downy mildew, white rot and Botrytis neck rot are far and away the most important. Considered in conjunction with the routine intelligence reports they indicated, among other things, that in two seasons when downy mildew was not generally above normal, but when neck rot was above normal, at least one-quarter of the onion acreage was affected to a greater or less extent by downy mildew; that about one-sixth of 12,000 acres devoted to onions was contaminated with the white rot parasite; and that one-tenth of the stored crop was destroyed by neck rot. Taking the 1943 average yield of 7·3 tons per acre, this means that in that year nearly 9000 tons of onions, worth about £270,000 at current prices, were lost from neck rot alone.

In 1941 there was a rather late, long drawn-out but fairly severe epidemic of potato blight with conditions favouring tuber losses on a considerable scale. Tuber infection was well above normal and caused some concern. To obtain accurate information it was decided to make a survey of potato clamps in the following autumn.

Now conditions in 1942 were rather different. Blight developed late but fairly rapidly and, as usual under such conditions, tuber losses in the clamp were not excessive. Judged from routine information they were, in fact, about normal. When a random sampling of the clamps was taken in November in many parts of England and Wales it was found that blight was responsible for an average loss of about 3% of the tubers. On the basis of present acreages and yields, this means rather more than 200,000 tons; enough to keep Birmingham, with its million inhabitants, supplied with potatoes for a year and a quarter, or roughly equivalent to the total output of potatoes from Devon in 1948.

One of the most helpful local surveys has been that of *Didymella* stem rot of tomato carried out by I. F. Storey in Yorkshire for the last four years. He took as his criterion the number of plants actually killed by *Didymella* at the time of inspection in late August or early September. In 1945, the peak year for this disease, he inspected 112 houses on thirty-one different holdings and found that approximately 7% of the 300,000 plants involved were killed. That meant a loss of about forty-five tons of fruit on those few holdings alone, or about £6000 in terms of retail prices then prevailing. And to this must be added the decreased value of yields from many other plants infected but not killed. So far I have carefully refrained from hypothetical figures, but cannot resist pointing out that what was true of Yorkshire in 1945 was true of most other districts and if, on the average, instead of 7% only 1% of plants had been killed under glass in England and Wales, this would have meant the loss of 1200 tons of fruit worth £160,000 at retail prices. In 1946 and 1947 there was a decided falling-off in the severity of the disease in Yorkshire, as elsewhere, and only about 2% of the plants were actually killed.

It is a happy circumstance that research workers are now directing their attention more and more to the economic significance of the diseases they study. The individual or the small team are able to employ more elaborate and more time-consuming methods which can, moreover, be adapted to serve particular ends. I propose merely to give one or two examples to illustrate the method and, incidentally, to protest against the misuse of results so obtained. E. C. Large (1945) who, with A. Beaumont, did invaluable work during the war in promoting potato spraying in the south-west, combined his successful demonstrations with careful observations on the prolongation of useful growth of the haulm by spraying, the time at which it occurred in relation to the development of the crop, and the ultimate effect on yield. He showed in no uncertain fashion, over a number of seasons, that in Devon and Cornwall, where blight commonly stops the growth of the plants in the middle of their most productive period, two sprayings will give, on the average, a gain of well over two tons per acre—on crops from certified seed and in reasonably good soil. He made it quite clear that the same result could not be expected, and in fact was not obtained, with poor seed, in poor soil, or where blight did not cause premature destruction of the haulm. By linking the trials with the blight curves to which I have already alluded it is possible to deduce that in a few other parts of the country, where

the curves might show the same relation to the course of blight development as in the south-west, spraying would give much the same result. But over much, and perhaps most of the country, we know that quite different 'blight curves' are or would be obtained. In many districts blight comes too late, in most years, for protective spraying to be worth while as a general practice. The work in the south-west was sound plant pathology: it showed *why* a mean increase of two tons per acre was obtained from spraying in that district, and pointed very clearly to the need for equally careful survey and trial work before anything could be said about the gain to be expected from spraying elsewhere. But in this age of headlines there has been more than a tendency to ignore the qualifications so rightly made, and merely to blazon the fact that by spraying your potatoes you can increase yields by over two tons per acre—irrespective of district, seed or soil. In the long run such unqualified statements do a great disservice to plant pathology, especially when they creep into text-books on the subject. These experiments in the south-west provide a good example of the kind of work which seems to me appropriate to the N.A.A.S. specialist plant pathologists, or field research officers as they might well be called.

Another investigation, in which laboratory and field research has been happily combined, is that by Watson, Watson & Hull (1946) on the factors affecting the loss of yield of sugar beet caused by beet yellows virus. Between 1933 and 1944 there were at least four years in which severe epidemics of yellows occurred, and the average yield of sugar beet in Great Britain for those years was 8 tons per acre, whereas in the other years the average yield was 9·6 tons per acre. That in itself, though a pointer, is not conclusive. But experimental work showed that plants which become infected as early as June or July lose half or more of their potential yield of sugar: indeed, it was shown that 1000 plants will lose about 10 lb. of potential yield of sugar every week after the appearance of symptoms. Put in another way, if a single 20-acre field becomes badly infected with yellows in mid-July, the amount of sugar lost from that field alone would equal the present sugar rations of 1000 people for 12 months.

A further example shows how standard methods of recording diseases can be adapted to meet specific research problems. I have already expressed the opinion that brown rot of apples is a problem of first-rate importance, and it is a disease for which a remedy is urgently needed. Unpublished results obtained by M. H. Moore, and kindly placed at my disposal, give a clue to the damage actually caused by it, and indicate that the remedy is partly one of controlling insect pests. Having devised a suitable method for assessing the loss, he showed that over three consecutive seasons an average of about 10% of the fruits in his research plots became infected on the trees that were given a complete spray schedule. On the trees that were not given scab sprays there was a further 10%, and on those not sprayed for codling moth a further 10–12%. So that theoretically 30% of the fruits in that particular orchard would have been attacked by brown rot had no spraying at all been carried out. And to this would have to be added a variable but appreciable value for contact

infections that did not occur in these trials because in the method employed infected fruits were regularly picked from the trees.

The significance of what may appear to be quite minor diseases must certainly not be overlooked. The brown rusts of wheat (*Puccinia triticina* Erikss.) and barley (*P. hordei* Otth) are among the many diseases commonly said to be of no economic importance, but the truth of this remains to be seen. In one instance at least we now have evidence to the contrary. The success achieved by the potato health certification scheme in controlling leaf roll and rugose mosaic has simultaneously brought into prominence the relative importance of virus *X*, hitherto regarded as of very little economic importance. This virus, however, occurs throughout commercial stocks of most varieties, and Bawden, Kassanis & Roberts (1948) have shown that the various strains of it reduce yields in amounts varying from 5 to 24%. They have estimated—perhaps a little rashly—that in Britain alone some 700,000 acres of potatoes are infected annually with virus *X*, and that because of this, yields are being reduced by more than 500,000 tons a year.

When contemplating the significance of plant diseases one can scarcely fail to be impressed by what appears to be a marked difference in the response of the producer to problems of disease. Though there are exceptions, it seems fairly obvious—and is perhaps only to be expected—that with crops having a relatively low value per unit area, the application of advances in plant pathology usually comes about as a result of encouragement or pressure from without, whereas with crops having a relatively high value per unit area it is the producer who applies the pressure and is impatient for fresh knowledge. It by no means follows, therefore, that the producer, the plant pathologist, and the economist, see eye to eye on the importance of disease. That is why I included cereal troubles with some hesitation among my forty principal diseases. Cereal-seed disinfection has proved a valuable weapon, and has come to be accepted as such, but this was achieved more by exhorting the producer than by satisfying his demand. For many years epidemics of mildew, foot rots, and yellow rust have come and gone with little more than gentle flutterings, as though they did not matter, while major diseases like chocolate spot, club root and clover rot have been tacitly accepted as necessary evils, and have at most received the fitful attention of plant pathologists. It is to the fruit orchards, and especially to the apple orchards, that one has turned for evidence of the producer's intense desire to benefit from the results of research. It is the hop farmer, with his few and costly acres, who has taken the initiative, and the flower grower who has been fully alive to the fact that an epidemic of disease might cost him dearly—the carnation grower who needs no pressure from outside to rebuild his houses if by so doing he can prevent the worst dangers of wilt; the narcissus grower who began to apply the treatment for basal rot almost before the details were published, and the gladiolus grower who now waits impatiently to be told how to avoid the corm rots that are ruining many of his valuable stocks. Fortunately, there are signs that the tide has already turned in the farming world, for in attempting to solve problems of food production one of the foremost

aims should be to increase production, not so much by doubling or trebling the acreages devoted to a crop in order to offset low average yields, as by ensuring the highest possible yield from the smallest acreage required to meet the demand. If and when that happy stage is reached, the agricultural farmer will be just as anxious about the state of health of his crops as is the producer of fruit and flowers, and the plant pathologist will find his rightful place in influencing the practices of good husbandry.

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THE MOVEMENT OF TOBACCO MOSAIC VIRUSES AND POTATO VIRUS *X* THROUGH TOMATO PLANTS

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(With 2 Text-figures)

Tomato aucuba mosaic virus, tobacco mosaic virus and potato virus *X* took 3·5–4, 5 and 3 days respectively to move from inoculated tomato leaflets into the petioles and stems.

On reaching the stem each virus usually first moved downward, but in some plants both upward and downward movement occurred simultaneously and in a few upward movement occurred first.

All three viruses travelled through the stem at approximately the same rate. Each was capable of travelling more than 80 cm. during the first 12 hr. after entering the stem, giving a minimal average rate of about 8 cm. per hr.

Uninfected pieces of stem invariably occurred between infected pieces. Maximum lengths of stem through which virus particles had apparently passed without causing infection, were 44·5, 49 and 39 cm. for the three viruses.

Introduction

The rates at which different viruses have been found to move in plants when causing systemic infections are recorded in the Appendix, from which it seems at first sight that different viruses must move in different manners and probably take different paths. However, it will be seen that the techniques used by different workers have also varied widely, and many of the apparent differences might result from this rather than from anything more fundamental. Most work has been done by infecting a plant at one site and then finding at what distance from the infection site the virus is detectable after a given time. The Appendix shows that the methods of introducing virus have varied from grafting and colonizing with infective vectors, to inoculating with plant extracts, differences that might well determine whether movement could start immediately or not. So, too, the methods of testing for the presence of virus in parts remote from the infection sites have varied, and whereas some were probably sensitive enough to detect small quantities of virus, others almost certainly were not. Hence the slower rates recorded may be far below those at which viruses travel through plants, for the time taken to become detectable at remote sites may be occupied largely, not by travelling the measured distance, but by a delay before movement begins and another after reaching the remote region during which virus multiplies sufficiently to become detectable.

There is already evidence of three such phases with tobacco mosaic virus (Böning, 1928; Holmes, 1930; Samuel, 1934; Kunkel, 1939). After infection there

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is an incubation period during which virus multiplies locally and there is no spread to remote parts. Such spread seems to occur only when the virus has reached the vascular tissues, when it soon travels long distances, though its presence is not detectable until multiplication has occurred in the newly invaded parts. The work described in the present paper was done to gain further information on the periods occupied by these three phases, to determine the rate and direction of movement when virus first enters the stems, and to compare tobacco mosaic with other viruses.

Materials and methods

Two strains of tobacco mosaic virus, namely, tomato aucuba mosaic and Johnson's ordinary tobacco mosaic, and a strain of potato virus *X* were used. All experiments were made in tomato (*Lycopersicon esculentum*), var. Kondine Red. Plants were grown in 7 in. pots and were inoculated when they were about 65 cm. high. Only plants that were uniform in size and appearance were used in any one experiment. A diagram was made of each plant to record the position of each leaf, length of the petiole of the inoculated leaf and the height of the plant (Fig. 1). All plants used in one test were inoculated within a few minutes of one another and to retain uniformity between different experiments all inoculations were made either between 9 and 9.30 a.m. or between 9 and 9.30 p.m. The inoculum was sap freshly expressed from systemically infected young leaves.

The technique used for inoculation, sampling and testing the sample pieces for presence of virus, was essentially the same as that described by Samuel (1934). The terminal leaflet of a leaf about midway up the stem of each plant was rubbed with the inoculum and at intervals the plants were cut with a sterilized knife into standardized pieces. The petiole was cut into three equal pieces (*x*, *y* and *z*), and six internodes, three (*1*, *2* and *3*) above and three (*a*, *b* and *c*) below the point of attachment of the inoculated leaf, were cut from the stem, *1* and *a*, being the internodes immediately adjoining the inoculated leaf (Fig. 1). Each piece was then halved, and the half nearer the inoculated leaflet was tested immediately for presence of virus by macerating it with 2 c.c. of water and inoculating the extract to test plants (*Immediate Inoculation* or 'II' tests); the other half was stored in sterile test-tubes (Holmes, 1930; Samuel, 1934) and similarly tested after 10 days' incubation (*Test-tube Incubation* or 'TI' tests). The bases of the plants which remained after sampling were kept for 6 weeks to see whether they developed symptoms and virus had travelled into them.

Nicotiana glutinosa was used as test plant for tomato aucuba mosaic and tobacco mosaic viruses, and *N. tabacum*, var. White Burley for potato virus *X*. The number of local necrotic lesions produced was recorded, to obtain some information on the virus concentration in the infected pieces.

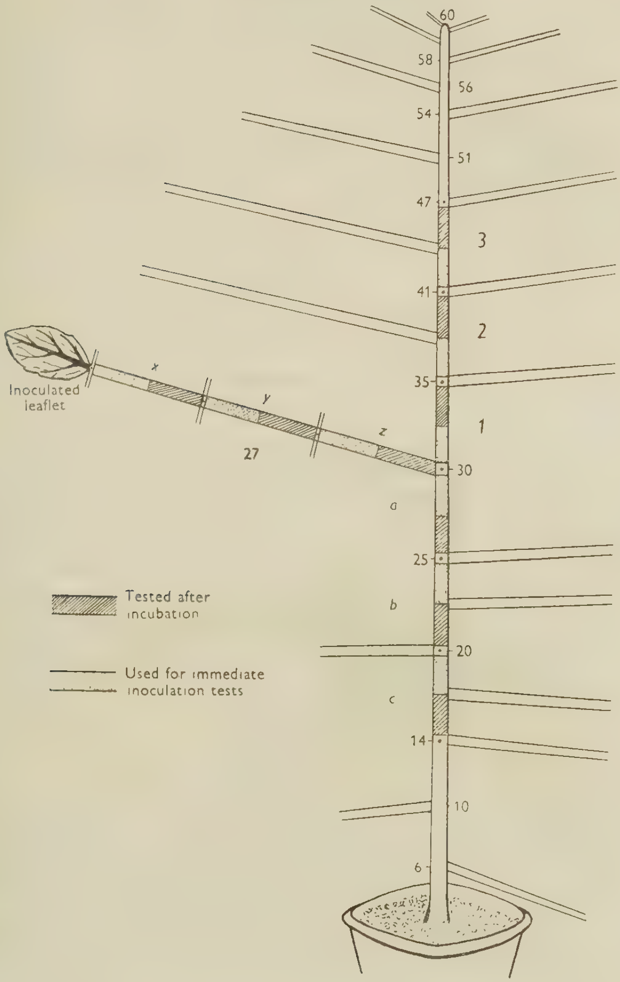

Fig. 1. Diagram of a tomato plant showing the plan of sampling, the height of each leaf on the stem and the total height of the plant in centimetres. Positions *a*, *b*, *c*, *1*, *2* and *3*, show the portions of stem internodes, and *x*, *y* and *z* those of the rachis of inoculated leaf, tested for presence of virus. Each portion was divided into halves, one of which was used for 'Immediate Inoculation' test and the other was tested for virus after incubation for 10 days.

Experimental results

(1) *Tomato aucuba mosaic virus*

Holmes (1930) could not demonstrate the presence of virus in uninoculated parts of plants until symptoms appeared on the apical leaves, and he concluded that, to produce a detectable amount of virus in regions of the inoculated leaf not more than an inch or so from the site of inoculation, required approximately as long as for virus to reach the extremities of the plant. Samuel (1934) also found that no virus could be detected in samples tested on the day of cutting, but he showed that some must have been infected, because equivalent samples incubated for 7 days and then tested contained much virus. These results have been fully confirmed in our experiments, when virus has regularly been demonstrable in stem pieces after incubation but not before. The obvious reason for this is that, at the time of sampling, the pieces contain too little virus for transmission to test plants, and that this multiplies during incubation. It was possible, however, that a considerable amount of virus might be present but, for some reason, possibly because of the occurrence of inhibitors of infectivity, it might not be transmissible to the test plants. Such non-infective virus often precipitates normally with virus antiserum, and to check this possibility precipitin tests using virus antisera were made on some extracts of petioles and stems clarified by heating up to 60° C. and centrifuging. The precipitin test gave essentially similar results to infectivity tests, and showed that only pieces of stem which gave infective extracts also precipitated specifically with virus antisera. Neither test was sensitive enough to detect virus immediately after it had entered a sample piece, but only after multiplying there for 2–3 days. The infectivity test proved more sensitive than precipitin tests, which were discontinued.

The results of tests with the incubated sample pieces are summarized in Table 1, which includes data from two experiments carried out in the months of September and November when the average temperature was 70° F. and the mean hours of sunshine per day during the experiments were 4·87 and 3·33 respectively. Plants were first sampled 3 days after inoculation, and thereafter at 12 hr. intervals up to the 10th day. In all, twenty-eight plants were inoculated and tested.

The data in Table 1 show that in two plants (nos. 5 and 6) the virus had moved from the inoculated leaflet within $3\frac{1}{2}$ days after inoculation, but in most movement occurred on the fourth day. None of the samples from plants (nos. 1–4) sampled during the first 3 days contained virus. In ten plants (nos. 5–8, 15, 16, 19 and 22–24) one or more of the samples were apparently virus-free, though other samples farther from the inoculated leaf were infected, suggesting that virus particles had moved through some portions of the plant without causing infection there. None of the sample pieces from plant no. 5 was infected, but the virus had moved down to the plant base, a total distance of 44·5 cm. without infecting the intervening portions. Plant no. 14 affords a striking contrast to no. 5, for the virus did not infect the base of the plant, although it was present in all the sample pieces tested. In plants nos. 5

and 6 movement occurred first towards the roots, but in most others the virus had moved both up and down in the stem within 12 hr. of first entering it. In none of the plants tested had the virus moved only upward. In plant no. 10 the virus moved a total distance of 94 cm. within 12 hr., or at the rate of 7·83 cm. per hour.

TABLE 1. *Incubation period of aucuba mosaic virus and its subsequent distribution at various intervals as shown by tests on incubated samples*

		Petiole			Stem internodes				
					Upper		Lower		
Plant no.	Interval in days	*x*	*y*	*z*	*2*	*1*	*a*	*b*	Plant bases
1–4	3	0	0	0	0	0	0	0	Healthy
5	3½	0	0	0	0	0	0	0	Infected
6	3½	0	0	0	0	0	22	32	Infected
7	4	17	0	8	0	0	0	0	Plant died
8	4	0	0	72	3	16	27	29	Plant died
9	4	250	47	300	350	400	540	602	Infected
10	4	286	560	392	328	350	400	522	Infected
11	4½	500	36	450	350	400	500	550	Infected
12	4½	500	450	360	300	450	500	650	Infected
13	5	98	350	550	500	450	550	600	Infected
14	5	7	2	44	5	8	6	2	Healthy
15	5	100	29	64	10	0	115	60	Plant died
16	5	45	49	67	50	0	50	94	Plant died
17	5½	462	300	450	270	528	672	700	Infected
18	5½	450	324	350	300	450	540	625	Infected
19	6	92	59	50	85	0	10	93	Plant died
20	6	100	118	100	106	120	210	240	Plant died
21–28	6½–10	+	+	+	+	+	+	+	Infected

+, positive infection.

(2) *Tobacco mosaic virus*

Samuel (1934) used 90 cm. high tomato plants in his experiments and found that tobacco virus moved out of the inoculated leaflet on the 4th day following inoculation. Kunkel (1939) used plants of an average height of 34 cm. and found that the virus moved out of the inoculated leaflet as early as 44 hr. after inoculation. In a preliminary experiment with this virus using plants of an average height of 92·5 cm. which were sampled at various intervals up to 5½ days after inoculation, no virus was detected in the inoculated samples and the plant bases also all remained uninfected. Thus it seemed that no movement occurred from the inoculated leaflet in 5½ days. In four other experiments done with this virus between April and August, plants of an average height of 65 cm. were used; the mean maximum temperature ranged from 74·6 to 87·8° F. and the minimum from 51 to 59° F., and the mean daily hours of sunshine were 1·67, 4·6, 5·88 and 5·88, for the duration of the four experiments. The plants were cut for sample pieces from the second day after inoculation up to the 8th day at intervals of 12 hr. The results of tests on the incubated samples are given in Table 2.

Table 2. *Incubation period of tobacco mosaic virus and its subsequent distribution at various intervals as shown by tests on incubated samples*

					Stem internodes						
	Interval	Petiole			Upper			Lower			
Plant no.	in days	*x*	*y*	*z*	*3*	*2*	*1*	*a*	*b*	*c*	Plant bases
1–10	2–4	0	0	0	0	0	0	0	0	0	Healthy
11–14	4½	0	0	0	0	0	0	0	0	0	Healthy
15	5	169	0	0	—	0	0	206	0	—	Infected
16–20	5	0	0	0	0	0	0	0	0	0	Healthy
21	5	341	222	160	—	133	0	0	186	—	Infected
22	5	0	4	0	—	17	12	4	0	—	Infected
23	5	91	0	0	—	0	30	80	3	—	Plant died
24	5	0	0	0	—	5	26	95	0	—	Healthy
25	5	0	1	0	—	0	0	0	0	—	Infected
26	5½	300	223	500	544	412	450	420	350	400	Infected
27	5½	1	0	30	8	38	0	0	0	0	Healthy
28	5½	196	318	378	—	66	207	360	11	—	Infected
29	5½	103	271	264	—	0	0	388	47	—	Infected
30	5½	80	R	10	—	257	240	350	434	—	Infected
31	5½	192	2	4	—	417	0	817	0	—	Infected
32	5½	164	250	R	—	300	420	520	575	—	Infected
33	5½	100	107	R	—	420	450	600	0	—	Healthy
34	6	0	0	0	0	0	0	0	0	0	Infected
35	6	150	140	100	202	400	490	314	300	213	Infected
36	6	182	396	390	—	279	350	460	520	—	Infected
37	6	167	86	100	—	326	450	138	388	—	Infected
38	6	8	28	0	—	33	0	0	0	—	Healthy
39	6	0	0	0	0	0	0	0	0	0	Healthy
40	6	106	15	500	—	233	490	530	600	—	Infected
41	6½	140	174	31	260	210	235	500	350	450	Infected
42	6½	218	306	149	—	110	111	227	192	—	Infected
43	6½	122	141	151	—	75	189	222	550	—	Infected
44	7	9	R	3	79	4	5	12	8	12	Infected
45	7	466	232	265	—	187	214	450	500	—	Infected
46	7	468	147	261	—	136	172	140	240	—	Infected
47	7½	184	193	232	—	42	83	124	168	—	Infected
48	8	194	188	227	—	106	146	160	150	—	Infected

R, sample piece spoiled.

Virus did not move out of inoculated leaflets until the 5th day after inoculation, when movement occurred in six of eleven plants sampled; in four of these (nos. 21–24) virus moved both up and down in the stem and in two (nos. 15 and 25) it moved only downward. Of twenty-three plants cut and tested between 5½ and 8 days after inoculation, virus apparently had not moved from the inoculated leaflet of no. 39 cut on the 6th day; in nos. 27 and 38 it had moved in the stem only upward, in nos. 29 and 34 only downward and in the other eighteen plants it had moved in both directions. In plant no. 33 the virus moved only a short distance downward but failed to infect the base, suggesting that the initial movement in this plant was mainly upward, as also in plant no. 24. Samuel (1934) concluded that, on entering the stem, virus first moved downward, and that the tops of plants usually became

infected via the roots. When fruit trusses were developing above the inoculated leaf, however, he found that virus movement occurred in their direction. Neither plant no. 33 nor no. 24 had any fruit trusses and it seems that in these the virus did not first pass to the roots. Virus moved down but not up in four plants (nos. 15, 25, 29 and 34) and up but not down in two (nos. 27 and 38). Plants nos. 15, 29 and 38 had fruit trusses developing at 19, 16·5 and 21 cm. respectively, above the inoculated leaf, and there were no fruit trusses on the other plants. These results suggest that the direction in which virus moves on first entering the stem is not necessarily determined by the presence or absence of developing fruit trusses, but probably depends on the translocation flow proceeding in the phloem vessels at the time the virus particles reach them.

The results with ten plants (nos. 15, 21–25, 27, 31, 34 and 38) suggest that the virus either passed through pieces of petiole, stem internodes, or both, without causing infection locally. In two plants (nos. 25 and 34) virus moved through total lengths of 49·0 and 45·5 cm. respectively, without infecting them. Further, in two plants (nos. 21 and 22) virus moved a total distance of 86 cm. in 12 hr., giving an average rate of 7·16 cm./hr.

The results of 'Immediate Inoculation' tests (Table 3) show that the presence of virus in petiole and stem internodes below the inoculated leaf was first detected on the 7th day (plants nos. 45 and 46), but the virus did not reach measurable concentrations in stem internodes above the point of attachment of the inoculated leaf even on the 8th day following inoculation (plant no. 48).

TABLE 3. *Occurrence of tobacco mosaic virus as shown by 'Immediate Inoculation' tests*

					Stem internodes			
		Petiole			Upper		Lower	
Plant no.*	Interval in days	*x*	*y*	*z*	*2*	*1*	*a*	*b*
1–35	2–6	0	0	0	0	0	0	0
36–42	6–6½	0	0	0	0	0	0	0
43	6½	2	3	0	0	0	0	0
44	7	0	0	0	0	0	0	0
45	7	9	5	4	0	0	1	1
46	7	19	13	14	0	0	18	5
47	7½	2	0	0	0	0	0	0
48	8	45	14	32	0	0	13	9

* Plant number corresponds to that in Table 2.

(3) *Potato virus X*

In the first experiment with potato virus *X*, sampling was started 3½ days after inoculation and continued up to 7½ days at intervals of 12 hr. 'TI' tests showed (results not tabulated) that the virus had already moved out of the inoculated leaflet and spread both up and down the stem within 3½ days. Four more experiments were made in July, August and September when the temperature ranged from 54 to

84·2° F. and the daily mean sunshine hours were 4·1, 2·85, 4·96 and 4·4 respectively, for the duration of the experiments. Sampling was begun 24 hr. after inoculation and continued up to the 5th day at intervals of 12 hr. The data given in Table 4 show that the virus moved out of the inoculated leaflets of four out of nine plants tested on the 3rd day; it had spread to all parts in plant no. 14, but moved only downward towards the roots in nos. 15–17. It appears that movement in plant no. 14 must have started sometime between $2\frac{1}{2}$ and 3 days after inoculation.

Table 4. *Incubation period of potato virus X and its subsequent distribution after different intervals following inoculation as shown by incubation tests*

					Stem internodes				
		Petiole			Upper		Lower		
Plant no.	Interval in days	*x*	*y*	*z*	*2*	*1*	*a*	*b*	Plant bases
1–2	1	0	0	0	0	0	0	0	Healthy
3–4	$1\frac{1}{2}$	0	0	0	0	0	0	0	Healthy
5–6	2	0	0	0	0	0	0	0	Healthy
7–8	$2\frac{1}{2}$	0	0	0	0	0	0	0	Healthy
9–13	3	0	0	0	0	0	0	0	Healthy
14	3	106	179	150	94	85	130	241	Infected
15	3	178	77	16	0	0	98	163	Infected
16	3	82	0	0	0	0	0	0	Infected
17	3	0	0	0	0	0	0	0	Infected
18–19	$3\frac{1}{2}$	0	0	0	0	0	0	0	Healthy
20	$3\frac{1}{2}$	0	0	0	0	0	420	0	Healthy
21	$3\frac{1}{2}$	220	78	0	0	0	0	180	Infected
22	$3\frac{1}{2}$	0	0	0	0	0	0	0	Plant died
23	$3\frac{1}{2}$	20	80	36	0	0	0	0	Healthy
24	$3\frac{1}{2}$	124	25	90	0	99	284	240	Infected
25	4	472	40	13	0	0	0	0	Infected
26	4	376	90	41	0	0	0	79	Infected
27	4	0	18	0	0	0	0	0	Healthy
28	4	0	0	0	0	0	0	0	Healthy
29	4	50	54	56	77	30	161	195	Infected
30	4	31	22	40	47	67	61	90	Infected
31	4	10	28	41	0	5	20	40	Infected
32	4	180	288	173	45	56	268	418	Infected
33	$4\frac{1}{2}$	138	148	248	47	79	155	164	Plant died
34	5	12	80	106	92	48	60	122	Infected
35	5	7	16	125	0	0	91	140	Infected

The data further show that of thirty-five plants virus did not move from the inoculated leaflets of seventeen (nos. 1–13, 18, 19, 22 and 28); it moved both up and down in eight (nos. 14, 24 and 29–34), only downward in plants nos. 15–17, 20, 21, 25, 26 and 35, and did not move into the stem internodes of plants nos. 23 and 27. The records of six plants (nos. 14, 29, 30 and 32–34), in which movement occurred, show that the virus had reached all sections. There was no plant in which movement was only upward. In several plants (nos. 16, 17, 20, 21 and 25–27) the virus passed through tissues without infecting them. In plant no. 17 the virus moved through

a total length of 39 cm. of tissue without infecting it. The virus moved in plant no. 14 a total distance of 96 cm. in 12 hr., giving an average rate of 8 cm./hr.

Direction of movement

The results show that so little virus moves at first from the inoculated leaflet that it cannot be detected by either infectivity (Table 3) or precipitin tests. Virus becomes detectable, it would seem, only when it infects tissues and multiplies in them, as when sample pieces are incubated (Table 2). The data also indicate that, in the earlier stages of systemic infection, virus does not move through petiole and stem along a concentration gradient (Table 3) in which concentration is highest near the inoculated leaflet and decreases steadily with increasing distance from it. On the contrary, it seems that virus particles enter the petiole and stem in an irregular and discontinuous manner, and the first particles may not become established and multiply until they have travelled long distances. This is the most logical explanation of apparently virus-free pieces of stem interspersed between infected pieces. Some samples which appeared virus-free when tested after incubation may have contained virus particles that for some reason or other failed to become established and multiply. Although this possibility cannot be excluded, it is reasonable to assume that those incubated pieces in which virus was first detected were also those to which virus particles first passed after leaving the inoculated leaflet. On this assumption the earliest stage of systemic invasion and multiplication of tobacco mosaic virus in tomato plants has been illustrated in Fig. 2. The presence of initial infecting virus in a sample is shown by one arrow, and increasing concentrations as estimated by lesion counts after incubation are represented by increasing numbers of arrows; four arrows is approximately the concentration that is infective to *N. glutinosa*.

Thus, 5 days after inoculation, virus was in sections *x* and *a* of plant no. 15 (Fig. 2A), in sections *x*, *y*, *z*, *b* and *2* of plant no. 21 (Fig. 2B) and in sections *y*, *a*, *1* and *2* of plant no. 22 (Fig. 2C) (Table 2). In plants nos. 15 and 21 more virus moved down the stem than up, whereas in plant no. 22 more moved up than down. On the 6th day, virus had reached almost all parts of the plants (plant no. 35, Table 2), and although in none had it reached a measurable concentration (Table 3), there was probably more in the petiole and the stem sections immediately below it than elsewhere. On the 7th day, virus concentration increased and in plants nos. 45 and 46 (Table 3) had reached a measurable concentration in sections *x*, *y*, *z*, *a* and *b*, but not in stem sections *1* and *2* (Fig. 2E).

The results indicate that the virus most often first moves down the stem, but there is no reason to assume that this is always so or that virus which later occurs in the upper parts has come from the roots. Often it is apparent that within a short period virus moves both up and down and sometimes there is upward movement before any downward movement.

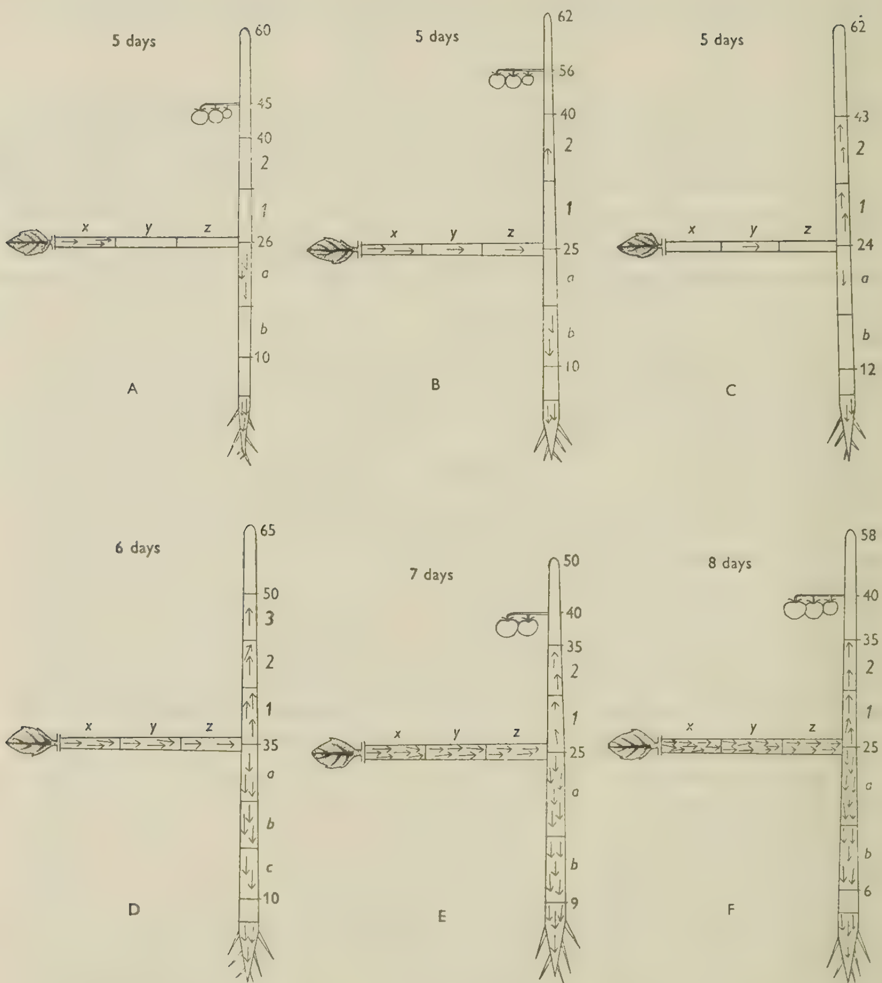

Fig. 2. Diagrammatic sketch of tomato plants showing the direction of movement of tobacco mosaic virus in them. The presence of an arrow or arrows in a section shows that the section had received virus particles, and the presence of at least four arrows in a section means that in that piece the virus had reached a measurable concentration. Interpretations are based on the results of the 'Test-tube Incubation' as well as 'Immediate Inoculation' tests of sample pieces. Inoculated leaflet shaded. A, plant no. 17; B, plant no. 21; C, plant no. 22; D, plant no. 35; E, plant no. 46; F, plant, no. 48 (Tables 3 and 4).

Discussion

The results recorded not only confirm observations made by Holmes (1930) and Samuel (1934) that there is a delay before tobacco mosaic virus passes from inoculated leaves into the petiole and stem, but show that this is also true of potato virus X; also that the delay, or incubation period, is different for different viruses and for different strains of one virus. During this period virus presumably multiplies at the entry points and passes from cell to cell in the leaf. The rate of movement is slow and is compatible with diffusion along a concentration gradient set up as the virus multiplies. This type of travel presumably was that measured by Uppal (1934). It probably continues until particles enter the phloem, where they encounter a stream of food materials in which they are transported rapidly to regions of food utilization and storage.

On entering the petiole and stem, aucuba mosaic, tobacco mosaic and potato virus X all moved at approximately the same rates, between 7 and 8 cm./hr. Such values could only be estimated from 12 hr. samplings, and actual rates, especially for short distances, may have exceeded these. Nevertheless, they are worth comparing with those recorded for other viruses in the Appendix. These rates are of the same order as found by Kunkel (1939) for tobacco mosaic, and the only rates much greater are those recorded for sugar-beet curly-top (Bennett, 1934) and maize-streak (Storey, 1928) viruses. Both of these were introduced by phloem-feeding insects and were probably introduced directly into the phloem, so that the actual infecting particles were in tissues where they could probably start to move away immediately. There can be no such certain timing of the entry of mechanically inoculable viruses into the phloem, hence accurate measurements of rates of movement are impossible. Those given above are certainly minimal and the maximal rates may well approximate to those found for curly-top and maize-streak viruses. Most workers, except Grainger (1933) and Caldwell (1934), have suggested that virus movement and translocation of elaborated food materials in plants are correlated and that long-distance transport occurs in the phloem. The evidence with curly top is particularly good, and the similarities between the speeds at which such different viruses as curly top, tobacco mosaic and potato X all move through stems suggests they all take a similar path.

On entering the stem, virus particles most often first move downward, no doubt because that is the direction in which the food stream is moving most often, but movement can happen in either direction.

In the initial phases of systemic invasion with each virus it is common for virus to be detectable in tissues remote from the infection site when intervening pieces of petiole and stem appear virus-free. Similar observations were made by Samuel (1934), Bennett (1934), Kunkel (1939) and Forbes & Mills (1945), and it seems that virus particles may travel through lengths of tissue without causing infection and multiplying there. Thus systemic invasion seems to occur from infections brought about at many different sites by particles carried over distances and not to any

steadily advancing autocatalytic reaction leading to continued production of virus (Grainger, 1933; Dixon, 1938). Virus-free sections most probably occur because virus particles that enter at one end all move through, but sometimes it may happen that particles are present but have not caused infection, as suggested by Caldwell (1931) and Kunkel (1939). However, all stem pieces become infected if the plant is left intact, so that they are susceptible and contain materials capable of supporting virus multiplication. The fact that such non-infected pieces occur is the strongest reason for concluding that movement over long distances occurs, not as a steady movement, but as erratic and separated dispersal of individual virus particles through the phloem to remote parts of the plant.

The author is indebted to Dr J. Henderson Smith for suggesting the problem and for his unfailing interest and advice given throughout the progress of the work.

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APPENDIX

Rates of movement of viruses in plants

Serial no.	Author and year	Virus	Host plant	Method of inoculation	Method of testing for presence of virus	Direction of movement	Distance travelled	In indicated time	Rate of movement in cm./hr.
1	Doolittle, 1920	Cucumber mosaic	Cucumber	Rubbing at the base of stem	Top leaf of main stem tested by inoculating to healthy plants	Up the stem	30 in.	5 days	0·635
2	Severin, 1924	Beet, curly-top	Beet seedlings	Leafhopper, *Eutettix tenellus*	Leaves severed at known distances after a known time	Down the leaf	7 in.	½ hr.	35·56
3	Murphy & M'Kay, 1926	Potato leaf-roll	Potato var. British Queen	Grafting at the top of stem	Tubers removed after intervals and grown	Down the stem	29 cm.	10 days	0·12
4	McCubbin & Smith, 1927	Tomato mosaic	Tomato	Injection at the top of main stem	Lateral branches layered and grown as cuttings	Down the stem	8–18 in.	10–15 days	0·084–0·127
5	McCubbin & Smith, 1930	Tomato mosaic	Tomato	Injection at the top of main stem	Lateral branches layered and grown as cuttings	Down the stem	—	—	1–2
6	Böning, 1928	Tobacco mosaic	Tobacco (leaf)	Leaf-tip inoculated by rubbing	Leaves severed at known distances and plants kept to develop symptoms	Down the leaf	13 cm.	2 days	0·27
7	Böning, 1928	Tobacco mosaic	Tomato (leaf)	Leaflet inoculated by rubbing	Leaves cut off and plants kept to develop symptoms	Out from the leaf	9 cm.	3 days	0·125
8	Böning, 1928	Tobacco mosaic	Tomato (stem)	Inoculum inserted in a wound at the top of stem	Stem pieces cut and tested by inoculating to healthy plants	Down the stem	(i) 12 cm. (ii) 20 cm.	4 days 4–5 days	(i) 0·125 (ii) 0·208–0·16
9	Böning, 1928	Tobacco mosaic	Tomato (stem)	Inoculum inserted in a wound at the base of stem	Stem pieces cut and tested by inoculating to healthy plants	Up the stem	(i) 12 cm. (ii) 25 cm.	3–4 days 4–5 days	0·16–0·125 0·26–0·208
10	Böning, 1928	Tomato streak	Tomato (leaf)	Inoculated leaflet by rubbing	Leaves cut off and plants kept to develop symptoms	Out from the leaf	9 cm.	4 days	0·09
11	Böning 1928	Tomato streak	Tomato (stem)	Inoculum inserted in wound at the top of stem	Stem pieces cut and tested by inoculating to healthy plants	Down the stem	(i) 12 cm. (ii) 20 cm.	6–7 days 5–6 days	0·083–0·071 0·16–0·14
12	Böning, 1928	Tomato streak	Tomato (stem)	Inoculum inserted in wound at the base of stem	Stem pieces cut and tested by inoculating to healthy plants	Up the stem	(i) 12 cm. (ii) 25 cm.	4–5 days 5 days	0·125–0·1 0·208
13	Storey, 1928	Maize streak	Maize	Leafhoppers, *Balcluthambila*	Leaf severed at a known distance and plants kept to develop symptoms	Down the leaf	40 cm.	2 hr.	20·00
14	Bennett, 1932	Red raspberry mosaic	Black raspberry	Aphid, *Amphorophora rubi*	—	Down the stem	49 in.	10 days	0·52
15	Bennett, 1934	Beet curly-top	Tobacco	Leafhopper, *Eutettix tenellus*	Stem cut into segments and rooted	Down the stem	24 in.	48 hr.	1·27
16	Bennett, 1934	Beet curly-top	Sugar beet	Leafhoppers feeding on cotyledons	Cotyledons removed at measured distance and plants kept to develop disease	Out of the cotyledon	30 in.	1 hr.	76·2
17	Bennett, 1934	Beet curly-top	Sugar beet	Leafhopper feeding at the tip of leaves	Leaves severed at distances from 1 to 10 in. at known time and plants kept to develop symptoms of disease	Out of the leaf	6 in.	6 min.	152·4
18	Uppal, 1934	Tobacco mosaic	*Nicotiana sylvestris*	Dorsal side of leaves inoculated by rubbing	Lower epidermis tested by inoculating to *Phaseolus vulgaris*	Through the leaf thickness	7–8μ	1 hr.	0·0008
19	Kunkel, 1939	Tobacco mosaic	Tomato	A leaf midway on stem inoculated by rubbing	Sample pieces tested by inoculating to *Nicotiana glutinosa*	Both up and down in the plant	14 in.	2 hr.	17·78

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ASPERMY—A NEW VIRUS DISEASE OF THE TOMATO

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(With 1 Text-figure)

'Aspermy' is suggested for the name of a virus disease of tomato apparently distinct from any previously described. The virus was transmitted by *Myzus persicae* from infected to healthy tobacco plants, but not from or to other hosts. Some properties of the virus are described together with the symptoms it causes in various hosts.

In the summer of 1944 a previously undescribed disease of the tomato was observed by one of us (J.C.) in a crop of outdoor tomatoes of the variety Moneymaker in the gardens of this College. The infected plants were distorted and stunted with well-developed proliferations and the yield of marketable fruit from the infected plants was very small. The distribution of the disease in the crop suggested that it was insect carried and that a nearby stock of chrysanthemums had served as a source of the infection. The causative agent was found to be a virus and a brief note (Blencowe & Caldwell, 1946) was published in 1946 calling attention to the disease which has since been reported from various areas, usually in market gardens with mixed crops.

The purpose of the present paper is to set on record more detailed observations on the disease and on the causal agent, and to indicate some general considerations which arise from its study.

The symptoms of the disease in the tomato (*Lycopersicon esculentum*)

The disease seems to affect the growing point of the main stem and often completely inhibits it, the consequent growth of the axillary shoots giving the plant a characteristic 'bushy' appearance. Many axillary shoots develop abortive apices and further branching takes place. The leaves are somewhat distorted and develop a marked dark green mottling, while proliferations occur on the upper surfaces of the rachis of the leaves at the base of the leaflets. If the plant is 'stopped', as is the normal practice in commercial holdings, the proliferations develop into actively growing shoots and these add to the bizarre appearance of the plant.

The production of fruits by diseased plants is severely curtailed. These are the main effects:

(*a*) The fruit already set when the plant became infected develop normally and nearby leaves show no symptoms.

(*b*) The number of fruits set after infection is usually reduced and the fruits themselves are normally very small, though this is not always so, and are frequently distorted.

(*c*) Seed formation in the fruits set after infection is completely suppressed. Even when seed is formed on a diseased plant, the amount of viable seed in those fruits formed at about the time of the infection is very small.

Experimental transmission

Tests for seed transmission

All the fruits from eleven plants of diseased tomato plants (Variety Stonor's Moneymaker) were collected in October 1944, and all the seeds recovered and sown. The green fruits were ripened indoors and the seed from them collected and sown later. As a control ten healthy fruits from plants of the same variety grown beside the diseased plants were harvested and the seed taken from them and sown at the same time. The details are given in Table 1.

Table 1

Plant	No. of fruit	Average no. of seed per fruit	Germination (%)
Diseased: 1st sowing	39	13·6	30·7
2nd sowing	57	8·1	34·7
Healthy (both sowings)	10	128·7	77·6

All the seedlings were normal and healthy from both diseased and healthy parents. There was no evidence that the virus was transmitted to the seedling (cf. Caldwell, 1934). This point is dealt with in greater detail later. It should be noticed that many diseased fruits were devoid of seed and most of the seed came from a relatively small number of apparently 'normal' fruit. It is reasonable to assume that these were 'set' before infection took place.

Sap transmission and host range

The virus is sap-transmissible to tomato plants but 100% infection is rarely obtained unless an abrasive such as Celite is sprinkled on the leaves before inoculation. The incubation period is normally 18–21 days, and the symptoms are much reduced in plants grown in a glasshouse—this effect appears to be associated with temperature, as outdoor plants in the height of summer show much less well-marked symptoms than do the same plants during cooler weather. Inoculations have also been made in *Nicotiana tabacum* and *N. glutinosa*. Symptoms appear in *N. tabacum* 8–16 days after inoculation—a yellow mosaic developing on the young leaves and diffuse chlorotic blotches on the inoculated leaves. As the young leaves enlarge, the yellow mosaic becomes less pronounced and a dark green blotching and discontinuous vein banding appear approximating on occasion to a ring-spot pattern. The symptoms become less obvious on older plants, though decapitation of the plant results in the reappearance of the symptoms on the leaves of the axillary shoots.

Sap inoculation to plants of *N. glutinosa* produced systemic infection in 18–21 days. On this host there are no local lesions and the first symptoms appear on the growing leaves, which are distorted. This is followed by a dark green 'savoying' and sometimes by a necrosis of the leaf. Occasionally the distortion is so severe as to result in a strap-shaped structure. The flower buds are distorted and the flowers are abnormal.

Inoculation from *N. tabacum* and *N. glutinosa* into tomato plants produced the usual symptoms in that host.

Inoculations from diseased tomato and tobacco plants into *N. sylvestris*, *Hyoscyamus niger*, *Solanum nodiflorum*, *S. nigrum*, *Datura stramonium* and *Cucumis sativa* have produced no symptoms, nor has it so far been possible to recover the virus from these species.

Inoculations from chrysanthemum plants of different varieties, growing near the tomatoes in which the disease was first noted, to *Nicotiana glutinosa* showed that some were infected. The symptoms caused in chrysanthemums are slight. There is a faint mottling with a general slight chlorosis and some reduction in the size of the plant and of its leaves. The difference in appearance between a diseased and healthy chrysanthemum plant of the same variety is sufficiently obvious to be recognized without being so characteristic as to allow of exact description.

The thermal inactivation period

Six portions of infective sap were taken, of which one was kept at room temperature and the others were maintained at a definite temperature for 10 min. in a water-bath. After treatment the sap was rapidly cooled and rubbed on to the leaves of healthy tobacco plants. The results of one such series are given in Table 2.

TABLE 2. *Thermal inactivation period*

Temp. (°C.)	Control	40	50	60	70	80
Plants (no. infected of 5 inoculated)	5	4	2	0	0	0

Ageing in vitro

Sap from diseased plants was left for varying periods at room temperature (20·5–21·5° C.) before being inoculated into batches of six tobacco plants. The results are tabulated in Table 3.

TABLE 3. *Effect of ageing on the virus*

Time	Control	6 hr.	12 hr.	24 hr.	48 hr.
No. of plants infected (total 6)	6	6	5	3	0

Aphid transmission of the virus under glasshouse conditions

No transmission has been obtained by aphids between tomato plants or from tobacco to tomato plants despite repeated attempts. The following species have been tested, unsuccessfully, as possible vectors, *Myzus persicae* (Sulz.), *Aphis fabae*

(Scop.), *Macrosiphoniella sanborni* (Gill.), *Macrosiphum euphorbiae* (Thomas). Further, no transmission was obtained using unidentified aphids, found actively infesting a diseased tomato plant in the field. On the other hand, *Myzus persicae* readily transmitted the virus from diseased to healthy tobacco plants.

Experiments were carried out to ascertain whether transmission of the virus was affected by the feeding time on infected material of the aphid and whether preliminary fasting had any effect on the transmission of the agent.

Adult apterous *Myzus persicae* were fed overnight on infected tobacco leaves and then placed on healthy tobacco plants for 24 hr. A similar group of insects was kept overnight in a cellophane-covered Petri dish before being fed on infected leaves for only 3 min. They were then allowed 24 hr. feeding on healthy tobacco. In each case five aphids per plant were used. The results are given in Table 4.

TABLE 4. *Effect of different infection-feeding times on virus transmission*

	No. of Aphids/plant	No. of plants	No. infected
Aphids fed overnight on infective source	5	12	3
Aphids starved before 3 min. infection feeding	5	10	8

Cross immunity tests with two strains of cucumber mosaic virus

Two strains of cucumber mosaic virus were obtained by the kindness of Dr K. S. Bhargava of Rothamsted Experimental Station. The first or 'spinach' strain produces necrotic local lesions on *Nicotiana tabacum* followed by systemic infection notable for the pronounced shortening and curling of the midrib accompanied by the buckling of the lamina at right angles to the midrib. The second or 'yellow' strain produces systemic symptoms on *N. tabacum* consisting of alternate yellow and dark green areas. The dark green patches are frequently distorted by blister-like malformations of the lamina (cf. Bhargava, 1948).

Sap inoculations of the tomato virus were made into healthy tobacco plants using Celite as an abrasive. Ten days later (3 days after systemic symptoms appeared) similar inoculations were made with the cucumber mosaic virus into the same leaves as before. The experimental arrangement was as follows:

Plants	First inoculation	Second inoculation
1–5	Tomato virus	'Spinach' strain virus
6–10	Tomato virus	'Yellow' strain virus
11–15	Tomato virus	None
16–20	None	'Spinach' strain virus
21–25	None	'Yellow' strain virus

Characteristic symptoms of cucumber mosaic developed in all the plants, whether previously infected with the tomato virus or not.

A further experiment was set up to ascertain whether infection with the tomato virus inhibited the development of local lesions on the leaves of tobacco plants when

the leaves were subsequently inoculated with the 'spinach' strain of cucumber mosaic virus.

Four half-leaves of each of nine tobacco plants were inoculated with the tomato virus using Celite as abrasive, the corresponding half-leaves being rubbed with distilled water and Celite. At varying intervals thereafter the whole leaf surface was rubbed with sap containing the cucumber mosaic virus. Counts made as the local lesions developed are summarized in Table 5, and show that the tomato virus had no recognizable effect on the formation of lesions by the cucumber mosaic virus.

Table 5. *Local lesions on tobacco leaves after inoculation with tomato virus and cucumber mosaic virus*

Plants	Interval before inoculation	H_2O + C.M.V.	T.V. + C.M.V.
1–3	3 days	300	307
4–6	6 days	153	149
7–9	9 days	69	70
		522	526

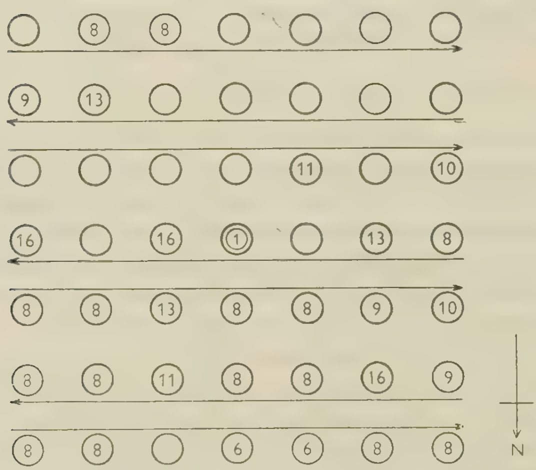

Fig. 1. For explanation see text.

Field transmission

An experiment was set up to examine the spread of the disease under field conditions. A square plot, of 49 tomato plants in seven rows each of seven plants of the variety Stonor's Moneymaker was set out in the second week of June, 1945. The centre plant had been artificially infected some weeks earlier. All the normal cultural operations of distributing, tying, etc., were carried out in the sequence indicated by the arrows in Fig. 1. Mechanical transmission of the disease on the hands or implements of the worker had an opportunity to occur, but should normally have spread along the rows and in the directions indicated by the arrows.

To simplify the presentation of the results the weeks of the tomato-growing season are numbered 1–17, i.e. from the second week in June to the first week in October inclusive. In the diagram the appearance of virus infection in a plant is indicated by the number of the week in which the disease was first noted. Thus, circles marked 8 indicate that infection was noted in the first week in August and so on. An examination of the diagram shows that the distribution of the infection is much more widespread than it should have been were only mechanical transmission responsible. Such a scattered spread is almost certainly attributable to the movement of some insect vector. The preponderance of infections on the north side of the plot, for example, cannot be attributed to infection from any nearby sources of infection outside the experimental plot, as a block of other tomato plants grown on that side showed only one instance of a late infection.

This observation confirms those made in others both before and since, namely, that in the field there is sporadic spread of the virus which suggests the activity of an insect vector, probably an aphid.

Discussion

The systemic symptoms produced in *Nicotiana glutinosa* and the absence of local lesions in the inoculated leaves of that plant indicate that the virus causing the disease under consideration is not that of ordinary tobacco mosaic, tomato spotted wilt or tomato bushy stunt (cf. Smith, 1937). The resistance of the virus to ageing *in vitro* and to heat also distinguishes it from these other viruses. The properties of the virus also distinguish it from that of tomato bunchy top which induces somewhat similar symptoms in the tomato (Smith, 1937).

The systemic symptoms in *N. tabacum* and *N. glutinosa* are very similar to those produced by cucumber mosaic virus (*Cucumis Virus I*) although the symptoms in the tomato are not markedly alike. (In Exp. IV it will be noted that the virus may be placed in the group of 'non-persistent' aphid transmitted viruses to which cucumber mosaic virus also belongs (Watson & Roberts, 1939).) To ascertain if there is any connexion between the two viruses, various experiments were carried out. Inoculations on to *Cucumis sativa* have not been successful with the virus under discussion.

There seems to be no immunological relationship between this virus and known strains of cucumber mosaic virus. The possibility that the tomato virus is a strain of cucumber mosaic virus may therefore be safely dismissed and it should be regarded as a new virus, *Lycopersicon* virus no. 7 (cf. Smith, 1937) or the virus of 'Aspermy' disease of tomato.

Kostoff (1933) studied the cytological aberrations in the reproductive organs of the plant induced as a result of infection with two viruses, viz. that of his so-called female sterility disease of tomato and that of tomato mosaic. He concluded that the abnormalities and sterility were associated with the effect of virus infection on the process of meiosis.

The non-production of seed in tomato plants infected with this virus is important in this connexion and indeed in the whole problem of the seed transmission of virus disease. Here is an instance in which virus infection prevents completely the production of seed and it would appear that the spore-producing cells are incapacitated by the virus (cf. Caldwell, 1934). The whole problem is of such importance that it will be fully dealt with in a subsequent paper.

The mode of spread of this virus in the field is still unsolved. There seems to be no doubt that the virus overwinters in the chrysanthemum, but its recognition in this host is difficult. The virus is almost certainly carried from infected chrysanthemums to tomato plants, but the identity of the vector has not yet been clearly established. The study of the vector and the manner of spread of the disease in the tomato crop is complicated by the fact that the tomato is a relatively unsatisfactory host for aphids. Although the field experiment has shown that the disease appears to spread within a plot it is possible, even probable, that the infection commonly spreads from a source outside the tomato crop. The early infections in June and July obviously produce the greatest loss of yield, as all the trusses tend to be formed after infection has taken place and all the fruits will be more or less unmarketable. Later infections affect the later trusses only. The roguing of suspected plants in the early part of the season is, therefore, well worth while and should keep the disease in check, or, at least, reduce the loss of crop to a minimum. Further work is clearly required on the recognition and eradication of diseased chrysanthemum stocks and on the identification of the vector of the virus.

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OBSERVATIONS ON A VIRUS DISEASE OF COWPEA IN TRINIDAD

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(With Plate 6)

A mosaic of cowpea and asparagus-bean (*Vigna unguiculata*) is common in Trinidad. The same virus sometimes attacks *Cajanus indicus*, *Crotalaria juncea*, *Glycine max*, *Phaseolus mungo* and *P. aureus*, usually when growing near to infected cowpeas. *Desmodium frutescens*, *Psophocarpus tetragonolobus*, *Sesbania speciosa*, *Vigna vexillata*, *Phaseolus trinervius* and varieties of *P. lunatus* are also susceptible to systemic infection. Most of the above give local lesions, which are the only symptoms produced on *Canavalia ensiformis* and *Dolichos lablab*. No infections were obtained on *Phaseolus vulgaris*, or on any plant outside the Leguminoseae.

The virus is seed-borne in asparagus-bean, but apparently not in tested cowpea varieties. The leaf beetle *Ceratoma ruficornis* is a vector, and is probably largely responsible for spread in the field; *Aphis medicaginis* seems unable to transmit the disease. The thermal inactivation-point of the virus is 66° C., its dilution end-point 1:100,000, and longevity *in vitro* over 20 days.

Introduction

Cowpea mosaic was recorded from Trinidad by Briant & Martyn (unpublished) in 1928. Certain information on the symptoms and host range has since been published (Dale, 1943), but this paper gives the results of a more detailed study of the virus. The disease is widespread on cowpea varieties, of which Black-eye and Gub-gub are most commonly grown, and on the related asparagus-bean, locally known as bodi. Some authorities classify cowpea as *Vigna sinensis*, asparagus-bean as *V. sesquipedalis* and catjang as *V. catjang*; others consider them varieties of *V. unguiculata* (Linn.) Walp., to which they are referred in this paper. The mosaic spreads rapidly, infection in a crop commonly reaching 100% within a few weeks of germination. There is no precise information on the effect of the virus on yield, but the infection of young seedlings causes a sudden growth check, though symptoms become less striking as diseased plants grow older. The light and dark green leaf-mottling varies in intensity for different cowpea and bodi varieties; on some of the latter a bright yellow mottling is produced.

Mosaics of cowpea were studied by Elliot (1921), Smith (1924), Gardner (1927), McLean (1941) and others in the U.S.A., where Snyder (1942) investigated an asparagus-bean mosaic. Similar diseases have been described from China (Yu, 1946), and India (Vasudeva, 1942; Capoor, Varma & Uppal, 1947), and are known in Africa, British Guiana and Jamaica.

Host range and symptoms

Mosaic symptoms occasionally occur on woolly pyrol (*Phaseolus mungo* L.), green gram (*P. aureus* Roxb.), soya bean (*Glycine max* (L.) Merr.) and sunn hemp (*Crotalaria juncea* L.), but only when growing near to infected cowpeas; and a similar disease of pigeon-pea (*Cajanus indicus* Spreng.) has been observed. Cross-inoculation experiments have shown that cowpea mosaic virus is responsible for the symptoms on each of these five crops. All can be readily infected by rubbing their leaves with juice from infected plants. Even without the aid of carborundum powder few inoculated plants fail to become diseased, though pigeon-pea is slightly less susceptible than the other hosts.

Chlorotic lesions, 2–4 mm. in diameter, appear on the inoculated leaves of many cowpea varieties. These may consist of alternating light and dark green rings (Pl. 6, fig. 1). When leaves are inoculated before attaining full size, the local lesions tend to coalesce, and vein-clearing or mottling of the whole or part of the leaf may develop. The next leaf to unfold usually shows pronounced vein-clearing (Pl. 6, fig. 2), which changes as the leaves expand to a fine-grained mosaic, made up of numerous dark green islands on a pale green background. Leaves developing subsequently show irregular yellowish and dark green mottling, accompanied by blistering of the lamina (Pl. 6, fig. 3). The final mosaic pattern is rather variable; coarse irregular mottling may be produced, but the more chlorotic coloration is usually associated with the veins, particularly at points of anastomosis. Sometimes, particularly under greenhouse conditions, reddish brown necrosis of the veins occurs, though this is not usually extensive. The sequence of symptoms described above can best be observed when inoculations are made with considerably diluted cowpea juice; otherwise local lesions are obscure and the vein-clearing phase less marked.

Young plants of *Phaseolus mungo* and *P. aureus* react to the virus by the appearance of chlorotic local lesions, followed by striking yellow vein-clearing of the first young leaf to unfold after infection. Severe green and yellowish mottling of subsequently developing leaves may be accompanied by blistering of the lamina. Infected soya beans are stunted, due to shortening of the internodes and petioles, while the leaves are darker green than normal and rather crinkled, the edges turning downwards. Axillary shoots proliferate and their small leaves may show light and dark green mottling, the latter being more pronounced under greenhouse conditions. Infected plants fail to set seed, or produce only a few pods. The field symptoms are very like those of soya-bean mosaic (Gardner & Kendrick, 1921). Leaf distortion and various types of mottling, accompanied by stunting of the plant, characterize the infection of *Crotalaria juncea* by cowpea mosaic virus. The leaf symptoms include variable mosaic patterns and chlorotic ring-spotting, and on leaves developing soon after infection localized necrosis may occur (Pl. 6, fig. 4*a*, *b*). The appearance of chlorotic local lesions precedes yellowish vein-clearing on pigeon-pea, the latter being followed by mottling or general chlorosis of the leaves. Artificial infection of young seedlings of this host often produces necrosis and death of the apical bud.

Although natural infection has not been observed, Lima bean (*Phaseolus lunatus* L.) and its large-seeded variety, King of the Garden (*P. lunatus* var. *macrocarpus* Benth.) can both be experimentally infected; without carborundum only 40% of the inoculated plants became diseased, and though with it most plants developed mosaic, these hosts seem to be less susceptible than the ones listed above. The symptoms on Lima bean resemble those on cowpea. *Vigna vexillata* (L.) A. Rich. and *Desmodium frutescens* (Jacq.) Schindl., native wild legumes, are susceptible to systemic infection; as are Jerusalem pea (*Phaseolus trinervius* Heyne), winged bean (*Psophocarpus tetragonolobus* DC.) and *Sesbania speciosa* Taub. ex Engl., introduced plants not commonly grown in Trinidad. When the simple leaves of *Canavalia ensiformis* DC. are inoculated, chocolate-brown, necrotic local lesions are produced. These are mainly about 1 mm. across, with a faint yellow halo. Virus has not been recovered from the symptomless, uninoculated leaves of this host. *Dolichos lablab* L. reacts in a similar manner, but the few local lesions produced do not become necrotic, resembling those on cowpea but less distinct.

Systemic symptoms, when produced, appear within 14 days of inoculation, this period being shorter for rapidly growing plants; for cowpea and sunn hemp it may be only 3 days. Local lesions are visible in 2–4 days. Young plants of systemically invaded species are more seriously affected than old ones and initial leaf symptoms are usually more severe than those shown later.

No infection was obtained on any of the following: *Phaseolus vulgaris* L.; *Nicotiana tabacum* L.; *N. glutinosa* L.; *Lycopersicum esculentum* Mill.; *Cucumis sativus* L. The rather limited host-range studies carried out suggest that the virus can infect many species of the Leguminosae. Almost all those tested are crop plants, but the family is very well represented in the Trinidad flora. Mosaic symptoms occur on wild species of *Crotalaria*, *Desmodium*, *Dioclea* and *Mucuna*, but attempts to transmit virus from these to cowpea were unsuccessful. Juice from Lima beans showing a mosaic, distinct from that described above, produced no symptoms on cowpeas; nor did the latter react to common bean mosaic virus, which attacks *Phaseolus vulgaris* in Trinidad.

Physical properties

The thermal inactivation-point, dilution end-point and longevity *in vitro* of cowpea mosaic were found in the usual manner, with recently infected cowpeas as a source of juice and cowpea seedlings, in the simple leaf stage, as test plants. Transmission occurred at dilutions up to 1:100,000, and 70% infection of test plants was obtained with juice stored for 20 days at 20° C. The results of thermal inactivation studies are given in Table 1.

Although local lesions are usually too indistinct for accurate counts, it was observed that a single lesion occurred on both plants infected by juice heated at 65° C., while for that held at 64° C. an average of over twenty lesions per plant was obtained. This suggests a high temperature coefficient of inactivation for the

virus, linking inactivation with protein destruction. In the dilution and longevity trials the number of local lesions, like the total of infected plants, decreased more gradually.

Table 1. *Inactivation temperature of cowpea mosaic* (10 *min. heating*)

Temp. (° C.)	Plants inoculated	Plants infected	Temp. (° C.)	Plants inoculated	Plants infected
30	50	50	64	50	41
60	50	50	65	50	2
62	50	48	66	50	0

Transmission

Gardner (1927) first reported the seed transmission of a cowpea mosaic and McLean (1941) showed that the degree of seed transmission varied, for different varieties, from 0 to 6·8%. Yu (1946) obtained a corresponding figure of 10·8% for the cowpea mosaic in China, Snyder (1942) demonstrated 37 % seed transmission for asparagus-bean mosaic and Capoor *et al.* (1947) found that 4 % of commercial seed of catjang gave mosaic-infected seedlings. In Trinidad, over 800 seedlings of the Gub-gub cowpea and 500 each of brown- and black-seeded varieties, raised from the seed of plants infected from an early stage, were free from the disease; but 8 % seed transmission was found in a sample of asparagus-bean seed, and its possible occurrence in untested cowpea varieties cannot be discounted. Although the virus seems to be the most infectious of those so far described on *Vigna unguiculata*, transmission by contact or during cultural operations can be of little importance.

Smith (1924) found the bean leaf beetle, *Ceratoma trifurcata* Forst., an efficient vector of a cowpea mosaic in the United States, and also transmitted the disease by inoculation with regurgitated juice or abdominal contents from individuals previously fed on infected plants. In Trinidad the leaf beetle *C. ruficornis* (Oliv.) attacks legumes, being especially destructive to cowpeas; and tests have shown that it is an efficient vector of cowpea mosaic. When single insects were allowed to feed for only 3 min. on diseased plants and transferred directly to healthy ones for a similar period of feeding, 30 % of the latter became infected. Higher figures were obtained by using more than one beetle to each test plant. Some individuals fed on diseased material for several hours were still infective 6 days later. The vector is closely related to that recorded by Smith (1924), whose results seem to have remained unconfirmed by other workers. There are few well-authenticated records of the regular transmission of a plant virus by biting insects, but Markham, Matthews & Smith (1948) have recently demonstrated it for turnip yellow-mosaic virus. Further experiments on the mechanism of beetle transmission of cowpea mosaic are in progress. There is no doubt that *C. ruficornis* is important in the field spread of the disease, and its preference for *Vigna unguiculata* may account for the much higher incidence of the virus in this host. The planting of successive crops of cowpea or asparagus-bean in close proximity should be avoided as far as possible, since

beetles from older plants migrate to younger ones, causing serious defoliation and rapid spread of the virus. The planting of other susceptible legumes adjacent to them is also undesirable. The control of the insect by dusting is not economic.

McLean (1941) transmitted his cowpea mosaic by four aphid species: *Macrosiphum solanifolii* Ashm., *M. pisi* Kalt., *Aphis gossypii* Glov., and an undetermined black aphid. Yu (1946) transmitted cowpea mosaic in China by *Aphis rumicis* L., *A. gossypii* and *Macrosiphum pisi*, the last-named also being a vector of asparagus-bean mosaic (Snyder, 1942). Mosaic of catjang was transmitted by *Aphis medicaginis* Koch., but not by *A. gossypii* (Capoor *et al.* 1947). The former sometimes attacks cowpeas in the field in Trinidad and may become abundant on greenhouse plants if not controlled; but repeated attempts to transmit cowpea mosaic by this insect were unsuccessful. Large numbers of aphids were used, and in some trials they were made to fast for several hours before feeding for relatively short periods on infected material and transference to healthy test plants. This method has given more efficient transmission with some aphid-transmitted non-persistent viruses. No other insects have been tested as vectors of cowpea mosaic in Trinidad.

Identity of the virus

Many descriptions of mosaic diseases of *Vigna unguiculata* have been brief; but McLean (1941), Snyder (1942), Yu (1946) and Capoor *et al.* (1947) gave fuller accounts, enabling comparisons to be made between their viruses and the one described above. The symptoms of the five are similar, but they differ in their host ranges and physical properties, the latter being compared in Table 2.

Table 2. *Comparison of properties of certain viruses of* Vigna unguiculata

Virus	Locality	Authority	Longevity *in vitro* (days)	Tolerance to dilution	Inactivation temp. (° C.)
Cowpea mosaic	U.S.A.	McLean, 1941	2–3	1 : 1,000	72–75
Cowpea mosaic	China	Yu, 1946	3–4	1 : 3,000	62–64
Cowpea mosaic	Trinidad		Over 20	1 : 100,000	65–66
Asparagus-bean mosaic	U.S.A.	Snyder, 1942	2–4	1 : 1,000	55–60
Catjang mosaic	India	Capoor *et al.* 1947	9–15	1 : 10,000	85–90

The virus described in this paper seems to have a more extensive host range than the other four. McLean transmitted his virus to the large-seeded Lima bean, but could not infect the small-seeded variety; Yu found the former susceptible to his cowpea mosaic but did not test the latter, which was presumably the one infected by the Indian workers; both are susceptible to the Trinidad virus. Soya bean, susceptible to the cowpea mosaic of Trinidad, seems to be immune to that of China and also to the asparagus-bean mosaic; while McLean failed to transmit his virus to *Phaseolus aureus*, a natural host of the Trinidad disease. *Canavalia ensiformis* has been infected by the Indian virus and the one described above, but whether the symptoms are similar is unknown. Asparagus-bean mosaic is the only one of the five

diseases which has been transmitted to *Phaseolus vulgaris*, and *P. angularis* (Willd.) W. F. Wight, susceptible to the cowpea mosaic of China, has not been tested elsewhere. *Cajanus indicus*, *Crotalaria juncea*, *Desmodium frutescens*, *Dolichos lablab*, *Phaseolus mungo*, *P. trinervius*, *Psophocarpus tetragonolobus*, *Sesbania speciosa* and *Vigna vexillata* have been infected only by the Trinidad virus. No host outside the Leguminoseae has been demonstrated for any of the five diseases. Raychaudhuri (1947) found that a mosaic of cowpea at Delhi was not transmissible to *Crotalaria juncea*, and that a mosaic of the latter in the same area did not infect cowpea. Price (1934) obtained two strains of cucumber mosaic that produced systemic mottling of Black-eye cowpeas, on which other strains of the virus produce only necrotic local lesions. These cowpea-mottling strains were isolated during experiments with yellow strains of cucumber mosaic. Natural infections have not been observed, but their symptoms were apparently similar to those of other cowpea mosaics.

The properties of viruses that have been recorded in *Vigna unguiculata* are too little known for any attempt to be made at relating them one with another. There is little indication that the one described in this paper is a strain of cucumber mosaic virus, and it may be unrelated to those which other workers have found to be aphid-transmitted. It is more likely to be the virus recorded by Smith (1924), which does not seem to have been studied during the last 25 years.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4*a*

Fig. 4*b*

DALE—*Observations on a virus disease of cowpea in Trinidad*

EXPLANATION OF PLATE 6

(Photographs by Dr H. Lees)

Fig. 1. Concentrically zoned, chlorotic local lesions on simple leaf of a black-seeded cowpea variety, inoculated with cowpea mosaic.

Fig. 2. Leaflet from trifoliate leaf of the same plant showing vein-clearing.

Fig. 3. Leaflet of cowpea showing mosaic and blistering produced by the virus.

Fig. 4. Leaves of *Crotalaria juncea* infected by cowpea mosaic: *a*, showing crinkling and mosaic; *b*, ring-spotting and necrosis.

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THE GROUPING AND OVERWINTERING OF *MYZUS PERSICAE* SULZ. ON *PRUNUS* SPECIES

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The aphid *Myzus persicae* Sulz. overwintered successfully on an almond-peach hybrid for three consecutive years. Experiments provided no evidence in favour of the theory that the olfactory sense is used by aphids to find their host, nor were flying aphids attracted to other *M. persicae* or to honeydew on the host. Having found their host, presumably by chance, alate gynoparae tended to associate in groups.

The aphid *Myzus persicae* Sulz. is known to be polyphagous in the viviparous stages, but the oviparae are much restricted in their choice of food. Hille Ris Lambers (1946) summarized the observations on the hibernation of *M. persicae* and concluded that the autumn alate gynoparae would produce oviparae on almost any *Prunus* sp. and that fertilized eggs would be laid. Hille Ris Lambers often found such eggs hatching in the spring, but the fundatrices failed to develop and give rise to colonies except on peach (*Prunus persica* Batsch). Doncaster & Gregory (1948) found *Myzus persicae* ovipositing only on cherry (*Prunus cerasus* L.) in addition to peach, but no colonies were found on cherry in the spring. Gorham (1941) described *P. nigra* Ait., the wild plum, as the common winter host of *Myzus persicae* in Canada, but this is not a European species. This paper records the almond-peach hybrid (*Prunus amygdalo-persica* (West) Rehd.) as a suitable host of hibernating *Myzus persicae*.

During the autumns of 1945 and 1946 oviparae and eggs of *M. persicae* were found on peach, nectarine, cherry and ornamental almond-peach in Harpenden. The following springs fundatrices were found on peach, nectarine and almond-peach, those on peach and nectarine giving rise to very large colonies, those on almond-peach to small colonies only. No fundatrices were found on cherry. During the spring of 1948 the colonies on almond-peach were as large as those on peach and nectarine, and numerous alatae were produced.

The distribution of alate *Myzus persicae* on their host

It has been noted (Profft, 1939, and others) that the autumn gynoparae of *M. persicae* are not distributed at random but congregate in groups on the leaves of the host tree. To find to what extent this occurred, the alatae on the leaves of an almond-peach tree were counted at intervals during the autumn of 1947. All the leaves on a number of branches taken at random were examined (Table 1).

The numbers of leaves with one, two, three, etc. alate *M. persicae* were recorded. The expected number of leaves with no aphids, had distribution been random, was calculated from the expression $e^{-m}n$, where m = mean number of aphids per leaf, n = number of leaves examined. In the last column of Table 1 the expected number

of leaves with no aphids is expressed as a percentage of the observed number. If the observed number of leaves with no aphids is greater than expected, this indicates grouping. The first record made showed that the aphids, which had recently begun to arrive on the tree, were distributed almost at random. Over the following fortnight, as more aphids arrived, aggregation became greater until as many as thirty-three were found on a leaf. The distribution of the aphids on the tree followed no apparent pattern. They were sometimes concentrated near the tips of long shoots, but by no means always (Table 2).

TABLE 1. *Counts of* Myzus persicae *on the leaves of* Prunus amygdalo-persica (*West*) *Rehd. at Harpenden*, 1947

Date (1947)	No. of leaves examined	No. of aphids per leaf: 0	1	2	3	4	5	6	7	8	9	10	11–15	16–25	26–33	Mean aphids per leaf	Expected no. of leaves with no aphids	Expected no. of leaves with no aphids as percentage of observed no.
		No. of leaves																
27. ix.	500	442	34	13	6	3	1	1	0	0	0	0	0	0	0	0·2	410 ± 8·4	92·8
30. ix.	200	138	31	13	7	1	3	1	4	0	0	1	1	0	0	0·8	92 ± 7·0	66·7
2. x.	200	113	36	16	7	7	2	5	2	0	2	2	7	1	0	1·6	42 ± 5·8	37·2
7. x.	106	46	18	14	5	5	2	5	1	2	0	2	3	3	0	2·4	9·6 ± 2·9	20·9
12. x.	100	26	8	10	4	7	3	5	2	6	3	3	10	10	3	6·5	0·14 ± 0·37	0·5

TABLE 2. *The numbers of aphids per five leaves, counting from the tips of long shoots*

Shoot tip	30. ix. 47	2. x. 47				7. x. 47		12. x. 47	
	4	8	5	3	12	17	24	83	38
	4	43	10	32	16	9	7	81	45
	4	21	12	4	0	32	9	32	24
	3	6	8	7	0	6	3	21	40
	4	10	1	13	1	2	1	39	17
	3	12	3	6	2	16	15	—	53
	3	10	4	1	0	—	3	—	6
	15	8	2	—	0	—	—	—	30
	7	4	—	—	—	—	—	—	31
	—	1	—	—	—	—	—	—	9
↓	—	—	—	—	—	—	—	—	46
Base	—	—	—	—	—	—	—	—	1

Many of the leaves without alatae were infested with young nymphs of oviparae, so gynoparae must have visited them and flown off again. This was shown to be so when twenty-eight leaves on a branch were inspected at 2 p.m. on 30 September and again at 9 a.m. the next day. Five leaves had fewer alatae, six had more and six had the same number at the time of the second count (eleven leaves had none on either occasion). The total numbers were 50 at 2 p.m. and 48 at 9 a.m. The number of leaves with x aphids was as follows:

$x=$	0	1	2	3	4	5	6
30. ix. 2 p.m.	12	5	1	2	5	1	2
1. x. 9 a.m.	13	3	2	2	6	1	1

The aphids tended to associate in groups near or on the midrib, often on the upper surface of the leaf near the tip, that is near the lower part of the leaf as it hung (usually bent under) and towards the outside of the tree.

Experiments on aggregation

Profft (1939) noted that alate *M. persicae* congregated in groups on peach leaves and stated that the aphids used olfactory organs to find their winter hosts; he postulated that the aphid first arriving might attract later arrivals by the scent of secreted honeydew, or of the material produced at the place of puncture.

To test this theory three sets of twenty small flasks were randomized on a glass-house bench, about 10 in. apart. Each contained water and was plugged with cotton-wool through which passed the stem of a peach leaf. One batch of leaves was from an uninfested tree, in another batch each leaf was infested with 1–4 aphids and in the third batch each leaf had been infested, but the aphids had been removed. About 500 alate *M. persicae* collected from peach and almond-peach trees were liberated in the glasshouse and each leaf was examined early every morning for the next 5 days (Table 3). Of the leaves originally without aphids, the clean ones were more heavily infested than those soiled with honey dew. The experiment was repeated with new batches of leaves and aphids. In neither experiment was there greater aggregation on leaves already infested. Apparently neither honeydew nor aphids attracted other aphids.

Table 3. *Total aphids on individual peach leaves in a glasshouse*

Leaves	Day 1	2	3	4	5	6
1st series: 20 clean	—	3	10	17	9	15
20 plus aphids	48	38	12	16	38	11
20 plus honeydew (Aphids removed)	—	4	5	3	2	2

Leaves	Day 1	2	3
2nd series: 20 clean	—	11	14
20 plus aphids	65	14	9

In another experiment four peach twigs, each with seven leaves, were exposed in a glasshouse with about 500 alate *M. persicae*. On two twigs the leaf glands were removed, and one twig with and one without glands were infested with aphids. The aphids per leaf were recorded at intervals (Table 4), usually at 9 a.m. and 5 p.m., the position of the twigs relative to one another being changed on each occasion. Again there was no evidence that the presence of aphids on a leaf attracted other aphids, nor that the leaf glands of the peach played any part in attracting aphids.

Another experiment was made to test if the aphids used the sense of smell to locate the plant. It is generally assumed that the olfactory sense organs are located

on the antennae, so the antennae were removed from half the test aphids. A peach twig with two leaves was held in a small conical flask inside a large covered glass cylinder in which a number of aphids was released. The flask was greased outside so that the aphids could only reach the leaves by flying. The aphids were released during the afternoon and those on the leaves were counted the next morning (Table 5). In the six batches 38 % of those with antennae were on the leaves when examined, 56 % of those without antennae were there. If the antennae are concerned with the olfactory sense it would seem that smell plays little part in host-finding.

TABLE 4. *Total aphids on seven leaves of peach twigs*

	Day									
	1	2		3		4		5	6	7
Twig	a.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	a.m.	a.m.
Aphids removed, glands present	—	0	3	4	7	7	8	10	13	19
Aphids present, glands present	38	39	43	42	41	43	44	44	48	47
Aphids removed, glands removed	—	1	3	4	7	8	8	7	11	15
Aphids present, glands removed	47	43	41	39	43	45	48	50	52	57

TABLE 5. *Alate* Myzus persicae, *with and without antennae, flying to peach*

	No. of aphids released		No. of aphids on leaves	
Batch	With antennae	Without antennae	With antennae	Without antennae
1	5	5	1	2
2	10	10	5	8
3	15	15	6	10
4	5	5	2	2
5	10	10	4	4
6	10	10	3	5
Totals	55	55	21	31

The non-random distribution of the alatae on their winter host might be the result of some leaves being more attractive than others, but in addition to congregating on certain leaves the aphids usually associate in one or more groups on those leaves. This grouping might result from the food in some portions of the leaf being more attractive than in others, from the photo-taxic or geo-taxic reactions of the insects, or from a gregarious sense exhibited by aphids when they meet. To test the last postulate, without possible interference from the other factors, experiments were done in darkness and in the absence of host-plants. A shallow glass dish, 4 in. in diameter, was divided by a cardboard partition into two and was covered with copper gauze on which rested an inverted shallow glass dish, 3 in. in diameter. One half of the lower dish contained 100 alate *M. persicae*, the other half was empty. Thirty-one alatae were placed in the upper dish. After half an hour in darkness there were nineteen aphids in the half of the upper dish over the empty part of the

lower, and twelve over the other aphids. This was repeated with the same result. The mass of aphids in the lower dish did not attract those in the upper dish through the gauze.

The same apparatus was used without aphids in the lower dish; the glass of the upper dish was marked into sectors and the number of aphids in each sector after a period in darkness was recorded. This was not difficult as almost all the aphids were on the glass sides or 'roof' of the dish, few remaining on the gauze. Experiments were made with four different batches of aphids (Table 6). There was significant non-random distribution when the aphids were left undisturbed for more than an hour. After shorter periods there was no significant grouping as shown by the χ^2 test.

Table 6. *Distribution of alate* Myzus persicae *in the dark*

Time (hr.)	No. of aphids	No. of sectors	χ^2	P
1	28	4	10·29	0·02
3	28	4	9·71	0·05
3	28	4	2·86	
13	26	4	2·00	
¼	27	4	1·89	
¼	27	4	0·71	
¼	27	4	0·71	
¼	27	4	1·89	
¼	27	4	1·30	
¼	27	4	4·85	
¼	27	4	4·56	
¼	27	4	2·19	
¼	27	4	3·67	
17	27	4	4·26	
2	75	6	12·76	0·05
½	75	6	3·80	
½	75	6	5·88	
½	75	6	5·24	
½	75	6	3·80	
½	75	6	2·52	
18	75	6	11·32	0·05
1	70	6	7·49	
2	69	6	4·65	
2	67	6	18·07	0·01
2	67	6	14·76	0·02
2	67	6	15·12	0·01
16	66	6	14·00	0·02
8	66	6	33·09	0·001

Periods	
Over 1 hr.	Under 1 hr.
$\Sigma n = 60$	$\Sigma n = 52$
$\Sigma\chi^2 = 160·38$	$\Sigma\chi^2 = 43·01$
(highly significant)	(not significant)

(n = degrees of freedom)

A similar test was made using 200 aphids in a 9 in. diameter dish covered with cellophane. The aphids begin to move after a few seconds exposure to light under

these experimental conditions, and when large numbers of aphids are used counts in the different sectors cannot be made before general movement begins. In this experiment, therefore, the distribution of the aphids was recorded by photograph; after the first exposure the aphids were left in darkness except for the flashes when subsequent photographs were taken after 2, 6 and 22 hr. The dish was divided into forty-eight equal areas by three concentric circles and twelve sectors. The aphids on the upper and lower surfaces of the dish could be distinguished by their shadows. Those on the side of the dish were counted separately. Table 7 gives the numbers

TABLE 7. *Analysis of photographs, numbers of aphids in different positions*

		Areas					
		1 centre	2	3	4 outside	Side of dish	Side ÷ 1·43 = same area as 1–4
A. In the light:	Top	4	3	6	36	43	30
	Bottom	56	31	9	12		
	Total	60	34	15	48		60
B. In the dark (2 hr.):	Top	14	33	26	73	43	30
	Bottom	3	5	0	3		
	Total	17	38	26	76		60
C. In the dark (6 hr.):	Top	13	19	34	77	49	34
	Bottom	4	0	1	3		
	Total	17	19	35	80		68
D. In the dark (22 hr.):	Top	25	25	38	48	51	36
	Bottom	2	1	3	7		
	Total	27	26	41	55		72
E. Mean in dark:	Top	17	26	33	66	48	34
	Bottom	3	2	1	4		
	Total	20	28	34	70		68

in the four equal areas demarked by the concentric circles and on the side of the dish. The area of the side was 1·43 times that of the areas on the top and bottom, so the number of aphids on the side was divided by 1·43 to give a number comparable to those recorded in the other divisions. In the light the majority of aphids were flying frequently, and the first photograph showed most to be on the bottom of the dish, where they normally land after a flight (Table 7A). In the dark, however, when flights do not take place but the aphids walk, very few were on the bottom (Table 7B, C, D). Analysis of those counted in the forty-eight divisions on the top showed no significant association in groups. The counts in the four areas marked by the circles and on the side showed a marked trend of aphid concentration from the centre to the side of the dish (Table 7E). This concentration of the aphids around the edge of the dish was noted in all the previous experiments. Any aphid walking in a straight line on the upper or lower surfaces will ultimately reach the edge, whereas those on the side can walk without leaving the side. Few crawling aphids

were seen on the bottom of the dish, so apparently most of those which reached it turned back. Thus a concentration resulted near the edge of the dish, perhaps increased by the tendency to associate shown in the previous experiments.

These few observations indicate that *M. persicae* probably does not find its host plant by smell, nor having found it does one aphid attract others. It is probable that the host is found by chance, and that having found it the aphids move about the tree until they find a favourable leaf on which they tend to remain. Conditions in the experimental dishes favoured continued movement, but there were no light or food stimuli to overshadow the tendency to associate; the fact that such a tendency was sometimes shown indicates that the grouping of alatae on *Prunus* may result from a number of aphids finding the tree by chance when flying, but tending to remain in association when they met during subsequent crawling.

The ornamental almond was kindly identified by the staff of the Herbarium, Royal Botanic Gardens, Kew.

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EFFECT OF PREVIOUS CROPS ON THE INCIDENCE OF EYESPOT ON WINTER WHEAT

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Surveys of winter wheat from 1939 to 1946 show that eyespot (*Cercosporella herpotrichoides* Fron.) occurs throughout Britain and that its incidence depends largely on previous cropping and on weather. Examination of 551 crops on land whose cropping for the previous 4 years was known showed that the incidence rose steadily with increasing numbers of preceding wheat and barley crops: where neither crop had been taken for 4 years the proportion of crops with more than 70 % infected straws was 2 %, rising to 45 % where three or four such crops had been taken and the average straws infected rose from 6 to 55 %.

The percentage infection to be expected in various groups of crops was calculated from previous cropping; it was compared with the actual infection and so used to assess the importance of other factors in determining the incidence of eyespot. High spring rainfall, early sowing and a dense plant increased incidence and low spring rainfall, late sowing and a thin plant reduced it.

Eyespot was not usually severe on newly ploughed grassland until the third or fourth crop of wheat, but under very wet conditions it was sometimes severe in the second crop.

Oats is much less susceptible than wheat or barley, but some crops were found with a third of their straws infected.

A brief survey of winter wheat in Holland suggested possible causes for the rise and fall of eyespot in recent years and for its present lower incidence there as compared with East Anglia.

Introduction

Surveys of winter wheat in Holland by Meijers (1933, 1937), Waal (1935) and v. Stegen (1936); in Germany by Bockmann (1939) and in Britain by Glynne (1942, 1946) and Storey (1947) show that although eyespot, caused by *Cercosporella herpotrichoides* Fron., is more often severe on wheat following wheat or barley than after other crops, it is sometimes severe on wheat grown on land where no wheat or barley has been grown for some years. Although extensive surveys were made in Holland and Germany, there are few quantitative estimates or published basic data from which calculations can be made of the influence on eyespot not only of crops grown in the previous year but also of those grown in earlier years.

The present paper gives the results of surveys of winter wheat in Britain for which eyespot intensity and the crops grown in the preceding 4 years have been recorded. The surveys include those made in 1945 and 1946 now described, scattered records obtained since 1939, and the results of similar surveys described by Glynne (1942, 1946) and by Storey (1947). Records for individual crops, except those already published by Glynne (1946, table 2), have been deposited at the British Museum (Natural History), Cromwell Road, London, S.W. 7. The data thus obtained from

551 crops in many parts of Britain are used to estimate quantitatively the effect of wheat or barley crops grown in the previous 4 years on the intensity of eyespot. The frequency with which different degrees of infection occur after different types of cropping is determined and the mean percentage of infected straws. The latter figures are used to compensate for differences in pre-cropping of differently classified groups of crops and so to measure the relative effects of other factors on eyespot.

These results are compared with those obtained in Holland in 1946 in an attempt to throw light on the reasons for the decline in the severity of eyespot reported there in the last decade.

1945–6 SURVEYS

Methods

Crops were surveyed in July or August (except fourteen in Scotland in June 1946). The kind of crop grown in each of the 4 preceding years was recorded with other data when available. Estimations were made of the percentage area lodged, the percentage straws straggled and those showing whiteheads and the cause of each condition determined by examination of the bases of the straws. Counts of 50–100 straws taken from ten random spots in upright and ten in lodged areas showed the numbers with slight and severe eyespot lesions, take-all, sharp eyespot and other foot-rotting pests and diseases. Crops were classified into those having 0, 1–5, 6–20, 21–50, 51–70, 71–100 % straws infected by eyespot. To determine the mean percentage straws infected in different groups of crops such as those examined in each survey, the mean percentage infection of each crop (e.g. 60 % for the infection class 51–70 %) is taken and the sum of these means divided by the number of crops.

Most of the crops surveyed were in the better wheat areas and include only a few on light sandy soils; in most surveys there was a slight bias in favour of land recently cropped with wheat or barley, and a strong bias in this direction in the surveys made in 1946 in East Anglia and the Midlands, in the scattered records 1939–45 and in those made by Storey in Yorks 1944–6.

Lodging. Of the area inspected in East Anglia 7 % was lodged by eyespot and 4 % non-parasitically in 1945, compared with 12 % by eyespot and 4 % non-parasitically in 1941. The survey in East Anglia and the Midlands in 1946, which included an exceptionally high proportion of heavily infected crops, showed 35 % of the area lodged by eyespot, 2 % non-parasitically.

Table 1 shows that lodging, which increased with the percentage straws infected, occurred with lower eyespot intensity in the wet season of 1946 than in the drier season of 1945. In both years crops with more than 70 % straws infected had more than half their total areas lodged.

TABLE 1. *Percentage area lodged related to percentage straws infected by eyespot*

Crops of winter wheat surveyed in East Anglia and Midlands

		Percentage straws infected by eyespot					
	Year	0	1–5	6–20	21–50	51–70	71–100
No. of crops	1945	33	20	24	19	6	12
No. of crops	1946	3	2	1	6	6	21
Percentage area lodged	1945	7	1	5	2	14	58
Percentage area lodged	1946	0	5	1	22	18	54

Straggling. In East Anglia in 1945 less than 1 % of the straws were straggled (fallen) in sixty-seven, 1–5 % in twenty-two and 6–20 % in two of the 105 crops examined. In 1946 there was more lodging and therefore fewer standing crops in which straggled straws could be observed. It exceeded 5 % in only one crop in which as many as 50 % of the straws were straggled by eyespot. In both seasons eyespot was the chief cause of straggling, though brown foot rot, sharp eyespot, wheat stem sawfly and Hessian fly sometimes caused straggling of a few straws.

Whiteheads. Whiteheads were less common than straggled straws. In East Anglia in 1945 less than 1 % of the ears were affected in thirty-two and 1–5 % in four crops; they were caused by eyespot in twenty-four, take-all in ten, brown foot rot in five and other agencies in three crops, more than one causal agent often occurring in the same crop. In 1946 take-all was found in thirteen crops, then too ripe to distinguish the whiteheads which were probably present; less than 5 % whiteheads was noted in twelve, and 10 % in one of the forty-five crops examined and they were mostly caused by eyespot.

Incidence of eyespot. The number of winter wheat crops with different degrees of infection is shown in Table 2.

TABLE 2. *Incidence of eyespot*

		No. of winter wheat crops						
		Percentage straws infected by eyespot						
Harvest year	Region	0	1–5	6–20	21–50	51–70	71–100	Total
1945	E. Anglia:							
	Hunts	8	0	2	3	0	0	13
	Lincs	2	5	10	2	1	1	21
	Cambs	8	3	2	3	0	3	19
	Essex	8	2	1	2	2	1	16
	Norfolk	4	7	8	9	3	5	36
	Total	30	17	23	19	6	10	105
1945	Devon	6	0	8	1	1	1	17
1946	Scotland: 3 counties	7	3	2	2	0	0	14
1946	England: 8 counties	4	2	3	7	6	24	46
1939–45	14 counties in England and 1 in Scotland	7	10	7	5	4	17	50

Eyespot has now been recorded from all wheat-growing districts of Britain in which critical examination has been made.

1939–46 SURVEYS

Frequency of eyespot after different types of cropping

Table 3 includes records from 551 crops inspected in all the surveys. The percentage of crops with more than 1, 20, 50 and 70 % straws infected (those with more than 1, 20 and 50 % each include all those in higher infection groups) after five

main types of cropping are shown. As the number of wheat or barley crops increased from none to three or four in the previous 4 years, the proportion of severely (over 70 %) infected crops rose from 2 to 45 %, the likelihood of severe eyespot thus increasing from one chance in fifty to nearly one in two.

TABLE 3. *Effect of preceding wheat or barley on percentage of crops having different degrees of infection by eyespot*

Wheat or barley crops in previous 4 years		Winter wheat crops inspected				
			Percentage having more straws infected than			
No.	Years	No.	1 %	20 %	50 %	70 %
0	—	116	40	6	3	2
1	3 or 4 years earlier	87	83	28	15	7
	1 or 2 years earlier	137	84	48	31	17
2	—	184	90	73	48	32
3 or 4	—	27	89	74	63	45

The occurrence of a few crops with more than 50 % straws infected on land where no wheat or barley had been grown for 4 or more years was attributed in one instance to spores blown in rain from adjacent heavily infected wheat crops on wet windswept land and in other instances, which occurred in Scotland in 1944, to the application of infected straw directly or in dung to the previous root crop. Although eyespot infection generally occurs below the level at which the straw is cut and so remains on affected land, under especially damp conditions the lesions may occur as high as 8–9 in. above soil level, thus infecting the straw which is removed from the field. Another instance of infection probably carried by straw was seen in wheat grown in Lincs on land which had carried no cereal crop for at least a hundred years; no eyespot could be found except in a small corner of the field which had received straw litter the previous year, where 30 % of the straws were infected.

Effect of previous wheat or barley crops on percentage eyespot

Although heavy infection occurred more often on land recently and frequently cropped with wheat or barley, widely different degrees of infection occurred after similar types of cropping. Table 4 gives, for all the surveys, the mean percentage straws infected in crops following each type of cropping with the mean values in the last column. The mean of 6 % infected straws where there had been no wheat or barley was much increased when one such crop had been grown. The more recently the susceptible crop had been grown, the greater was the increase, but there was so little difference when grown 1 rather than 2, or 3 rather than 4 years earlier that each pair has been treated as one cropping group having respectively 20 and 32 % straws infected. Although two susceptible crops appeared more effective in increasing infection when grown in successive than in alternate years, the differences were not consistent or statistically significant, so they have been treated as one cropping

group with a mean of 47 % straws infected. It rose to 55 % when three or four susceptible crops had been grown.

These figures have been used in a new way to estimate the importance of other factors on eyespot. Assuming that the mean percentage straws infected in all the crops examined after each type of cropping represents that occurring under average conditions, we can calculate the percentage likely to be infected in groups of crops classified in different ways. When actual infection exceeds (or falls short of) that calculated from pre-cropping, other factors must have been more (or less) than normally favourable to eyespot.

Effect of season

The actual mean percentage eyespot and that calculated from pre-cropping are recorded for each survey in the two lowest lines of Table 4. In Table 5 the regional surveys are arranged in order of deviation of actual from calculated infection, that is, in the order in which conditions other than pre-cropping became less favourable to eyespot. Moisture, likely to be one of the most important factors, is seldom deficient in winter, but dry periods in spring are likely to check infection. When less than 1 in. rain falls in a month this may be regarded as a period of drought. The mean rainfall figures for March and April from three to five recording stations in the regions surveyed are shown; those for May are omitted as they exceeded 1 in. in each survey. The deviation between the infection found and that calculated from pre-cropping was closely related to spring rainfall, the highest positive deviation occurring after rather heavy April rainfall and the lowest negative deviation in the only region where less than 1 in. had fallen both in March and April. The exceptionally low infection associated with high rainfall in North Wales in 1941 may have been due to rarity of sources of infection where there was little long-term arable land. No eyespot was found there in any of the wheat grown on recently ploughed land, while in other regions about half such crops had some infection.

Development of eyespot after grass

In southern England eyespot seemed to be seldom severe until the third or fourth wheat crop after grass, but in Scotland in 1944 it was sometimes severe in the second crop. The combined influence of pre-cropping and of moisture is illustrated by the different rates of development of eyespot after grass on three farms shown in Table 6. Rainfall at the recording station nearest each farm was more than 1 in. in May in each district, so is not included. In Warwick in 1945 less than 1 in. rain fell in both March and April, and eyespot was low in the second and third, but very severe with extensive lodging in the fourth wheat crop;* in the following year after more than 1 in. rain in both March and April, severe infection and lodging occurred in the third wheat crop. Similarly, in Northants a satisfactory wheat crop

* 22 and 20 cwt. of grain were obtained from the second and third and only 14 cwt. p./a., including an abnormally high proportion of tail corn, from the fourth wheat crop.

TABLE 4. *Mean percentage straws infected by eyespot after different types of cropping in different parts of Britain between 1939 and 1946*

1 (a) No. of wheat and barley crops in the preceding 4 yr.	1 (b) Year	1 (c) Cropping group	2 East Anglia 1941 No. of fields	2 East Anglia 1941 Mean % eyespot	2 East Anglia 1945 No.	2 East Anglia 1945 %	3 (a) Devon 1945 No.	3 (a) Devon 1945 %	3 (b) E.A. and M.* 1946 No.	3 (b) E.A. and M.* 1946 %	3 (c) Records 1939–45 No.	3 (c) Records 1939–45 %	4 Wales 1941 No.	4 Wales 1941 %	5 Scotland 1944 No.	5 Scotland 1944 %	5 Scotland 1946 No.	5 Scotland 1946 %	6 Yorkshire (recorded by I. F. Storey) 1944 No.	6 Yorkshire 1944 %	6 Yorkshire 1945 No.	6 Yorkshire 1945 %	6 Yorkshire 1946 No.	6 Yorkshire 1946 %	7 (a) (b) (c) All surveys: Total no. of crops	7 All surveys: Mean % eyespot	
0	—	1	8	4	21	6	2	0	2	1	5	3	18	0	46	11	8	2	0	—	2	1	4	1	116		6
1	4 years	2	2	28	2	0	0	—	2	49	0	—	0	—	12	23	2	8	4	4	3	8	4	18	31	20	20
	3 years		2	10	18	10	0	—	2	6	6	43	1	45	9	6	0	—	1	12	6	32	11	35	56	21	
	2 years	3	3	60	20	20	3	4	7	44	7	25	1	0	9	29	2	0	5	51	11	25	18	42	86	31	32
	1 year		0	—	8	42	4	12	2	60	5	4	0	—	28	33	0	—	2	30	2	48	0	—	51	33	
			7	35	48	20	7	9	13	42	18	25	2	28	58	28	4	4	12	28	22	27	33	40	224	27	
2	In alternate years	4	14	53	22	18	1	60	5	85	11	52	0	—	8	54	0	—	12	55	18	20	26	60	117	44	47
	In successive years		2	60	11	56	3	16	19	66	13	52	0	—	4	39	2	35	7	33	1	85	5	55	67	52	
			16	54	33	31	4	27	24	70	24	52	0	—	12	49	2	35	19	47	19	24	31	59	184		
3 or 4		5	0	—	3	32	4	28	7	67	3	85	0	—	5	75	0	—	1	0	1	60	3	36	27		55
Total no. of fields			31	—	105	—	17	—	46	—	50	—	20	—	121	—	14	—	32	—	44	—	71	—	551		
Actual mean % eyespot				37		21		16		58		39		2		25		7		38		25		44			
Probable % eyespot calculated from previous cropping				31		30		38		41		37		8		22		18		40		35		36			

* East Anglia and Midlands.

TABLE 5. *Spring rainfall related to percentage infection after compensating for differences in previous cropping*

Region	Harvest year	Deviation of actual from calculated mean % eyespot	Rain (in.) Mar.	Apr.	Total Mar.–Apr.
E. Anglia	1946	+17	1·08	2·42	3·50
Yorks	1946	+ 8	1·16	1·49	2·65
E. Anglia	1941	+ 6	3·18	1·56	4·74
Scotland	1944	+ 3	0·88	1·95	2·83
Yorks	1944	− 2	0·48	2·28	2·76
N. Wales	1941	− 6	2·78	1·80	4·58
E. Anglia	1945	− 9	0·93	1·06	1·99
Yorks	1945	−10	0·57	1·59	2·16
Scotland	1946	−11	1·64	0·46	2·10
Devon	1945	−22	0·79	0·76	1·55

TABLE 6. *Development of eyespot in winter wheat on ploughed-up grassland*

	Crops grown in harvest years				1945				1946			
						% straws infected	Rain (in.)			% straws infected	Rain (in.)	
County	1941	1942	1943	1944	Crop	eyespot	Mar.	Apr.	Crop	eyespot	Mar.	Apr.
Warwick	G	G	G	W	W	4	0·82	0·85	W	87	1·10	2·30
	G	G	G	W	W	4			—	—		
	G	O	W	W	W	32			—	—		
	G	W	W	W	W	95			—	—		
Acle, Norfolk	G	O	P	P	W	2	2·12	2·59	—	—		
	G	M	P	P	W	8			—	—		
	G	G	G	W	W	87			—	—		
	G	G	M	W	W	80			—	—		
Northants	G	W	W	Be	W	—	0·61	0·75	W	82	1·32	1·18
	G	O	W	Be	W	—			W	90		
	G	G	W	W	Be	—			W	90		

G = grass; O = oats; P = potatoes; M = mustard; Be = winter beans; W = wheat.

was reported after beans preceded by two wheat crops in 1945 after a dry March and April but in 1946 after heavier spring rain, the same crop sequence was followed by severe eyespot and lodging. At the Norfolk station on damp low-lying land, in 1945 rainfall was exceptionally high for that season with more than 2 in. in both March and April; severe infection and lodging occurred in the second wheat crop. Thus a high percentage infection with lodging was found in the second wheat crop after grass when the total March–April rainfall was 4·71 in., in the third when it was 3·40 in., and 2·50 in., and in the fourth when it was 1·67 in.

Effect of density

A few records of sowing rates were obtained, and in some other crops counts were made of the number of ears per foot. Fourteen crops sown with more than $2\frac{1}{2}$ bushels per acre, or having more than thirty ears per ft. had 28% of their total

area lodged, and 43 % infected by eyespot, which is 20 % higher than that calculated from pre-cropping. Eight crops sown at lower rates or having less than thirty ears per ft. showed no lodging and had only 8 % straws infected, 18 % less than that calculated, showing that conditions were more than normally favourable to eyespot in the dense crops and less so in the thinner crops. This was further illustrated by a field drilled with 2 bushels per acre which had 8 % straws infected, except in a small area accidentally drilled twice where infection amounted to 38 %.

Date of sowing

Decrease of eyespot with lateness of sowing and its deviation from that calculated from pre-cropping is shown in Table 7; positive deviations indicate conditions more favourable to eyespot than the average for crops sown in September and October, and negative deviations show them less than normally favourable in crops sown from November to spring.

TABLE 7. *Eyespot infection of winter wheat related to date of sowing*

Month sown	No. of crops	Mean % straws infected	Deviation from mean calculated from previous cropping
Sept.	4	72	+35
Oct.	56	47	+12
Nov.	24	22	− 9
Dec.–Spring	17	12	−14

Though not included in the systematic surveys a few crops of spring wheat were inspected. Exceptions to the usually low eyespot incidence in spring wheat were found in two crops following winter barley and wheat respectively which had as many as 56 and 75 % straws infected.

Other hosts

The susceptibility to eyespot of other hosts is important, not only because of the effect on the infected crops but also because of their effect as carriers of eyespot to subsequent wheat crops.

Eyespot in barley. Severe infection found in autumn-sown barley suggests that it is about as susceptible to eyespot as wheat. Spring-sown barley usually seems to escape serious infection in southern England, but is more affected in Scotland where, in 1944, seventeen out of eighteen crops were infected, and in 1946 eight out of twelve, including one, the third successive barley crop with 67 % straws infected.

As most barley is sown in spring and most wheat in autumn it seemed likely that on an average barley would be a less efficient carrier of eyespot than wheat. This was confirmed by the fact that of the winter wheat crops in the surveys here described ninety-one which were preceded by barley as the last susceptible crop, having had no wheat in the previous 2 years, had a mean of 32 % straws infected, which was 9 % less than that to be expected from previous cropping.

It may therefore be significant that the few wheat crops which were free from eyespot (Table 3) after three or four susceptible crops had all been preceded by barley and no wheat crop had been grown in the 2 preceding years.

Eyespot in oats. Eyespot was found affecting 26 % of the straws in a crop of winter oats at Rothamsted in 1945. Since then infection of both spring and winter oats has been reported from several counties in England and Scotland. Table 8 shows the previous cropping and infection of the oat crops inspected by the writers.

TABLE 8. *Eyespot in oats*

District	Year	Crop inspected	Preceding crops				Eyespot % straws infected
Rothamsted, Herts	1945	Winter oats	G	sO	W	W	26
(Delharding field)	1946	Winter oats	sO	W	W	wO	9
Acle, Norfolk	1945	Winter oats	G	W	W	W	33
Northants	1946	Spring oats	G	F	W	W	30

G = grass; sO = spring oats; wO = winter oats; F = fallow; W = wheat.

The mean percentage infection of these oat crops fell short of that to be expected in wheat crops after similar cropping by as much as 25 % indicating that some other condition, presumably host susceptibility, was much less than normally favourable to eyespot. This view is supported by infection experiments in which the fungus isolates from wheat, barley and oats, though they all infected each host, attacked oats much less severely than they attacked wheat or barley. Although it does not often seem to carry much eyespot, oats may have contributed to the high infection (75 %) found in a wheat crop after the crop sequence clover, wheat, winter oats, beans; volunteer oat plants (from the crop sown nearly 3 years earlier) having 28 % straws infected were found in the infected wheat crop.

Eyespot in grasses. The small amount of eyespot usually found on the first wheat crop after grass suggests that the British pasture grasses are seldom extensively infected. Oört (1936) thought that as diseased grasses are rarely found in Holland, their influence in spreading eyespot could not be great; both he and Sprague (1936) in America found lesions on a few wild grasses and succeeded in inoculating a larger number with *C. herpotrichoides*. In England isolations made from eyespot lesions on wild oats, *Avena ludoviciana*, growing in an infected wheat crop on Broadbalk field at Rothamsted, produced typical eyespot lesions when inoculated into wheat, but another fungus forming similar spores on *Agrostis alba* found in Yorks by Storey did not infect wheat. (A similar fungus was found later on *Agrostis* sp. by Glynne at Rothamsted.)

EYESPOT IN HOLLAND

The importance of eyespot in Europe was realized largely as a result of the work of Dutch investigators during the severe outbreaks which occurred in Holland from 1934 to 1938. As the disease no longer seems to be important there it is worth

considering the possible causes, though they cannot be determined with certainty, for the rise and fall of eyespot in Holland.

A government subsidy in 1932 had resulted in doubling the area of land under wheat and in almost doubling the percentage of arable land under wheat and barley. The resulting closer cropping of cereals was probably responsible for the high incidence of eyespot which was noted in 1934, the third year of increased cereal cultivation. Eyespot rose to a peak in the next 2 years, declined a little with slight decrease in the land under wheat in 1937 and 1938, fell to a low level in 1939, and does not seem to have been serious since, although there were only small fluctuations in the total area under wheat. But the proportion of wheat sown in spring, which averaged 15 % for the years 1932–8, rose suddenly in 1939 to 67 % and maintained an annual average from 1939 to 1945 of 49%. This must have been an important factor in reducing eyespot.

A survey in Holland in 1946 made it possible to compare wheat there with that in England in the same season. Less than 1 % of the wheat seen in Holland was lodged and about 12 % in part of southern England. Twenty-six winter wheat crops inspected in Holland (not including any on recently reclaimed land) had a mean of 14 % straws infected, which fell below that expected from previous cropping by 15 %, while infection in the crops inspected in East Anglia exceeded that expected from pre-cropping by 17 %. Lower April rain, which in parts of Holland fell below 1 in. and replacement of winter by spring wheat, were probably contributory factors but do not seem adequate to account for quite so large a difference in infection. It seemed likely that another factor reducing eyespot was the low seed rate and wider spacing of rows recorded respectively as $1\frac{1}{2}$–$2\frac{1}{2}$ with a mean of 2 bushels per acre and 7–12 in. in Holland as compared with 2–3 with a mean of 2·6 bushels per acre and 6–7 in. in East Anglia.

DISCUSSION

An estimate of the frequency of susceptible crops in any region is given by the proportion of the arable land devoted to wheat or barley. From 1936 to 1945 an annual mean of about 17 % was under wheat or barley in Holland, 30 % in England and Wales and more than 40 % in many of the eastern counties; it is not therefore surprising that eyespot appears frequently in these eastern counties. The likelihood of serious infection by eyespot on the better wheat land can be assessed from previous cropping (Table 3), and from other factors indicated above and used before sowing to assess the desirability for taking precautions to avoid loss; these include modifications in cropping such as the introduction of temporary leys, spring sowing, thin seeding, spraying with sulphuric acid and growing short-strawed varieties with added nitrogen.

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STUDIES IN THE DIAGNOSIS OF MINERAL DEFICIENCY

VI. THE COMPOSITION OF WEED LEAVES IN RELATION TO POTASSIUM DEFICIENCY IN BARLEY

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(With 1 Text-figure)

Leaves of five weed species (*Brassica sinapis*, *Cirsium arvense*, *Polygonum aviculare*, *P. convolvulus* and *Potentilla reptans*) from fertilizer trials on barley at four sites in Hampshire were analysed with a view to using their composition in forecasting the response of barley to fertilizers. The samples were gathered from as many of the 108 plots as possible, and were analysed spectrographically for calcium, iron, magnesium, manganese, potassium and sodium.

Marked differences in composition between the five species were recorded, the most noteworthy being the high sodium content of *Brassica sinapis* and *Cirsium arvense* and the high manganese content of *Potentilla reptans*. There were also marked differences between the four sites, but these were not uniform as between the different species, and often failed to agree with those observed for barley.

Superphosphate applications decreased the manganese content of the weeds in many cases, and increased their calcium content. Muriate of potash increased their potassium content, but tended to decrease that of magnesium and sodium. The only general effect of sulphate of ammonia on the composition of the weeds was a decrease in iron content.

Except in *Cirsium arvense*, the potassium content of weed leaves was correlated with that of barley on the same plot if differences within a site only were considered. Differences between sites were not correlated in this way. The correlation between potassium content of weed leaves and the response of barley to muriate of potash application was worthy of note only in *Polygonum convolvulus*, and even in this case the correlation of site differences did not reach significance. It is tentatively suggested that increases in the grain yield of barley as a result of muriate of potash application are likely to occur only where the leaves of *P. convolvulus* contain less than 1·83 % potassium.

Introduction

In a previous paper (Goodall, 1948*a*) an account was given of analyses of organs of barley plants from four fertilizer trials at four sites in Hampshire on soils thought to be deficient in potassium. It was found that there was a highly significant negative correlation between the potassium content of the untreated plants (particularly stems and older leaves) at the time of ear emergence and the increases in yield as a result of muriate of potash applications. Consequently, it should be possible to use such analytical data as the basis for a forecast of response to potassic

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fertilizers, i.e. for a diagnosis of potassium deficiency. But as the plants were sampled for analysis after the fertilizer application took place, and in fact too late for any such application to be effective, this diagnosis would be without practical value in respect of the crop sampled, and could be applied only to improving the nutrition of subsequent crops on the same site. To be of practical value in suggesting the fertilizer programme for a particular crop, a diagnostic method must be based on data obtainable before a fertilizer application would be made; it was therefore desirable to examine whether samples of barley plants could be replaced for this purpose by plant material present on the site before planting time, and the most promising source of such material appeared to be the common weeds of cultivation. If a correlation could be established between the potassium content of a weed species and the response of barley on the same soil to potassic fertilizers, analysis of weed samples taken before planting time could be used as a basis for determining the fertilizer treatment to be applied. With this possibility in mind, samples of common weeds were gathered from the four fertilizer trials mentioned, and analysed in the same way as those of the barley plants themselves.

The diagnostic analysis of weed samples is not a new procedure. Indeed, in 1862, Weinhold suggested that the composition of weeds might indicate the nutritional status of the soil on which they were growing, since a plant species would grow best on soil supplying nutrients in the same proportions as they were needed by the plant. He found some difficulty in fitting analytical data on arable weeds into this concept, but 2 years later (Weinhold, 1864) modified it to apply to undisturbed vegetation only, and used analyses of woodland plants to predict the performance of cereals if the land were subsequently brought under cultivation. The use of the method seems then to have lapsed for 40 years when Hall (1905), dissatisfied with the analysis of crop plants at harvest-time for diagnostic purposes, again suggested the analysis of weeds to forecast the fertilizer requirements of soils. He indicated that this was about to receive practical attention, but no results appear to have been published. In 1934, Haddock found that *Chenopodium album* on magnesium-deficient soil grew much better, and contained much more magnesium, on plots in which the deficiency had been remedied; he suggested that this species should be used as an indicator plant for magnesium, but whether his intention was to analyse the plants or merely to observe their growth is not clear. Teakle, Thomas & Turton (1941) investigated the possibility of using analyses of Cape weed (*Cryptostemma calendulaceum*) to indicate deficiency of copper for cereal cultivation, but their conclusions were negative. The present writer (Goodall, 1948*b*) analysed leaves of *Potentilla anserina* and *Cirsium arvense* from wheat fields under test for manganese deficiency; though the manganese contents of crop plants and of weeds were correlated, the data for the latter were not closely enough related to the response of the wheat to manganese sulphate sprays to be a promising basis for deficiency diagnosis.

Experimental procedure

Particulars have already been given (Goodall, 1948*a*) of the four fertilizer trials used for the present observations. They were designed as $3 \times 3 \times 3$ experiments, two degrees of freedom for the second-order interaction being confounded with block differences; treatments were 0, $\frac{3}{4}$ and $1\frac{1}{2}$ cwt. sulphate of ammonia, 0, $1\frac{1}{2}$ and 3 cwt. superphosphate and 0, $\frac{1}{2}$ and 1 cwt. muriate of potash per acre.

A first collection of weed samples was made on 14 and 15 June 1943, at the same time as the barley plants themselves were sampled. At this time the only weed at all generally distributed over the experimental plots was charlock (*Brassica sinapis*), and only this species was collected. A second series of samples was taken on 18 and 19 August, at which time four other species were sufficiently widespread to be worth collection, viz. black bindweed (*Polygonum convolvulus*), cinquefoil (*Potentilla reptans*), knotgrass (*Polygonum aviculare*) and field thistle (*Cirsium arvense*). In Table 1 is shown the number of plots at each site from which each weed species was collected.

Table 1. *Number of plots from which weed samples were collected*

	Chilbolton	Kimpton	Leckford	Snoddington	Total
Brassica sinapis	27	11	27	26	91
Cirsium arvense	26	0	3	2	31
Polygonum aviculare	25	4	27	2	58
P. convolvulus	27	2	25	24	78
Potentilla reptans	0	27	0	15	42

In the laboratory the leaves of these weed samples were removed from the stems, cleaned, dried and ground. They were subsequently analysed spectrographically for calcium, iron, magnesium, manganese, potassium and sodium by the technique already described (Goodall, 1948*b*).

Results

The analytical data were expressed as percentages of the element in question in the dry matter, and were analysed statistically within each species for those sites at which samples from all or most of the plots were available. The error estimates were examined by Bartlett's (1937) μ-test, and were found to differ from site to site only in magnesium content of knotgrass and manganese and potassium content of black bindweed. The significance of comparisons between sites was tested by the methods described by Yates & Cochran (1938).

Calcium. The mean content of calcium in each weed species at each site where it occurred is shown in Table 2. There are marked differences between the species in calcium content, i.e. charlock > thistle > black bindweed > knotgrass = cinquefoil (the number of comparisons on the same site is not sufficient to establish a difference between the last two); all are much greater than barley—a difference commonly found between dicotyledons and monocotyledons (cf. Collander, 1941). Certain site differences are apparent; the calcium content of charlock leaves was lower at Kimpton than at the other sites. Both thistle and knotgrass leaves contain more

calcium at Leckford than at Chilbolton. Other site differences are not significant. These results are not conspicuously in agreement with the barley analyses, in which for calcium content Chilbolton < Leckford < Kimpton = Snoddington; these differences between species may well be due to differences in rooting depth or in the seasonal course of development.

Significant increases in the calcium content of black bindweed and charlock were recorded at Snoddington and Leckford respectively as a result of superphosphate application at 3 cwt./acre (see Table 3), and the same tendency was observed in several other instances; this may doubtless be ascribed to the calcium content of the fertilizer. Apart from this, the only fertilizer effect observed was a highly significant

TABLE 2. *Mean calcium content (% of dry matter) of weed leaves at each site*

	Chilbolton	Kimpton	Leckford	Snoddington	All samples
Brassica sinapis	3·34	2·99	3·82	3·56	3·50
Cirsium arvense	2·74	—	4·25	3·02	2·90
Polygonum aviculare	1·95	2·21	2·25	1·74	2·10
P. convolvulus	2·23	2·88	2·40	2·21	2·29
Potentilla reptans	—	1·63	—	1·72	1·66
Barley (lower leaf blades)	0·29	0·58	0·37	0·59	0·44

TABLE 3. *Effect of superphosphate application on calcium content of weed leaves (% of dry matter)*

		Superphosphate applied (cwt./acre)			
Species	Site	0	1½	3	S.E.
Brassica sinapis	Chilbolton	3·31	3·14	3·56	0·27
	Leckford	3·39	4·37	3·68	0·27
	Snoddington	3·65	3·22	3·79	0·27
Cirsium arvense	Chilbolton	2·79	2·93	2·49	0·16
Polygonum aviculare	Chilbolton	1·96	1·93	1·96	0·13
	Leckford	2·22	2·19	2·35	0·13
P. convolvulus	Chilbolton	2·32	2·03	2·33	0·14
	Leckford	2·20	2·28	2·68	0·14
	Snoddington	2·23	2·01	2·39	0·14
Potentilla reptans	Kimpton	1·74	1·37	1·76	0·18

increase in the calcium content of thistle leaves at Chilbolton as a result of the application of muriate of potash. This increase is far too marked to be ignored (K_0: 2·20 % Ca, K_1: 2·91 % Ca, K_2: 3·10 % Ca; $F = 9{\cdot}02$, $P < 0{\cdot}01$), surprising as it is that such an effect should be confined to a single species.

Iron. The means for iron content are shown in Table 4. Certain differences among the species again become evident. Knotgrass, for instance, had a lower iron content than the other weed species. Comparisons between species are, however, complicated by the fact that their frequency varied from site to site. As regards site differences, Chilbolton gave consistently high values (the higher values for knotgrass at Kimpton and Snoddington are based on few samples), and three values at Kimpton were higher than those at Snoddington. The Leckford samples do not seem to take a consistent position in the series, charlock containing significantly

more iron than at Snoddington, while the black bindweed samples contained less. All samples contained more iron than those of barley leaves, and in the latter the site comparisons for iron content showed: Kimpton > Chilbolton = Leckford (the Snoddington values did not differ significantly from the other sites). In other words, the iron content of barley plants growing on different soils appears to bear little relation to that of the weed species tested from the same soils.

Sulphate of ammonia had the effect of reducing the iron content of the weed species (Table 5), though this effect reached significance only in the case of knotgrass. The effect of sulphate of ammonia on the iron content of the barley plants

Table 4. *Mean iron content (p.p.m. in dry matter) of weed leaves at each site*

	Chilbolton	Kimpton	Leckford	Snoddington	All samples
Brassica sinapis	702	553	504	447	552
Cirsium arvense	560	—	339	518	536
Polygonum aviculare	481	615	361	770	444
P. convolvulus	637	571	411	506	523
Potentilla reptans	—	620	—	533	589
Barley (lower leaf blades)	189	261	204	269	228

Table 5. *Effect of sulphate of ammonia on mean iron content (p.p.m. in dry matter) of weed leaves at Chilbolton*

	Sulphate of ammonia applied (cwt./acre)			
Species	0	$\frac{3}{4}$	$1\frac{1}{2}$	S.E.
Brassica sinapis	763	679	666	42
Cirsium arvense	629	549	502	48
Polygonum aviculare	539	485	419	24
P. convolvulus	641	678	593	36

Table 6. *Mean magnesium content (% in dry matter) of weed leaves at each site*

	Chilbolton	Kimpton	Leckford	Snoddington	All samples
Brassica sinapis	0·526	0·418	0·480	0·403	0·464
Cirsium arvense	0·388	—	0·292	0·358	0·377
Polygonum aviculare	0·449	0·694	0·416	0·265	0·444
P. convolvulus	0·580	0·455	0·458	0·453	0·499
Potentilla reptans	—	0·491	—	0·407	0·461
Barley (lower leaf blades)	0·215	0·258	0·216	0·211	0·225

was similar, but did not reach significance (N_0: 254, N_1: 250, N_2: 228; $F = 1·34$, $P > 0·20$). The only other significant effects of treatments on iron content of weeds were at Leckford, where that of black bindweed leaves was affected both by superphosphate (P_0: 409, P_1: 356, P_2: 467; $F = 4·09$, $P < 0·05$) and muriate of potash (K_0: 466, K_1: 412, K_2: 353; $F = 432$, $P < 0·05$). These results were not paralleled in other species or at other sites.

Magnesium. The means for magnesium content at the various sites are shown in Table 6. Taking the data as a whole, the species with the lowest magnesium content appears to be the thistle, knotgrass taking up an intermediate position (except at

Kimpton, where the high value is due to a single aberrant sample), and the other three species not differing greatly. All species contained substantially more magnesium than barley leaf blades, the average for which at all four sites was 0·225 %. As between sites, the magnesium content at Chilbolton tends to be higher than at the other sites, which do not differ significantly among themselves. Once again this site effect differs from that found for barley, in which the highest magnesium content occurred at Kimpton.

None of the treatments had significant effects on the magnesium content of the weed leaves. In the majority of comparisons, however, as in the case of barley, the

TABLE 7. *Effect of muriate of potash on magnesium content (% in dry matter) of weed leaves*

		Muriate of potash applied (cwt./acre)			
Species	Site	0	½	1	S.E.
Brassica sinapis	Chilbolton	0·608	0·504	0·466	0·068
	Leckford	0·460	0·480	0·500	0·068
	Snoddington	0·470	0·369	0·370	0·068
Cirsium arvense	Chilbolton	0·356	0·404	0·404	0·027
Polygonum aviculare	Chilbolton	0·549	0·412	0·384	0·035
	Leckford	0·450	0·379	0·420	0·069
P. convolvulus	Chilbolton	0·547	0·574	0·620	0·059
	Leckford	0·468	0·494	0·412	0·059
	Snoddington	0·473	0·502	0·384	0·059
Potentilla reptans	Kimpton	0·483	0·516	0·476	0·062

TABLE 8. *Mean manganese content (p.p.m. in dry matter) of weed leaves at each site*

	Chilbolton	Kimpton	Leckford	Snoddington	All samples
Brassica sinapis	290	262	270	228	263
Cirsium arvense	196	—	268	160	201
Polygonum aviculare	273	299	359	226	313
P. convolvulus	211	144	132	119	156
Potentilla reptans	—	784	—	483	676
Barley (lower leaf blades)	125	171	133	137	140

magnesium content is less in samples from plots receiving potassic fertilizer than in those from plots not receiving it (Table 7).

Manganese. Mean manganese content data are presented in Table 8. The manganese content of cinquefoil leaves appears to be outstandingly high, that of creeping thistle and black bindweed low, while charlock and knotgrass occupy intermediate positions. The mean manganese content of barley leaf blades is less than for the leaves of any of the five weed species. Snoddington has given consistently the lowest manganese content, but the differences among the other sites are inconsistent. Attention should be drawn to the comparison of Chilbolton with Leckford in respect of the two *Polygonum* species. In each the difference is highly

significant, but whereas for black bindweed the higher manganese content occurs at Chilbolton, for knotgrass it is at Kimpton. It would thus appear that, in these two related species, variations in manganese content from site to site may be determined by quite different factors. In the barley samples, site comparisons presented a different picture again, for the manganese content at Kimpton was high, while the other three sites showed only small differences.

A highly significant reduction in manganese content is evident on the Chilbolton plots to which superphosphate was applied (except for *P. aviculare*), and similar effects not reaching significance are also to be seen at other sites and in other species (Table 9). No other treatment effects were found. In barley, by contrast, muriate of potash decreased the manganese content and superphosphate had no effect.

TABLE 9. *Effect of superphosphate on manganese content (p.p.m. in dry matter) of weed leaves*

		Superphosphate applied (cwt./acre)			
Species	Site	0	1½	3	S.E.
Brassica sinapis	Chilbolton	298	302	269	16
	Leckford	292	276	243	16
	Snoddington	233	221	228	16
Cirsium arvense	Chilbolton	202	211	169	12
Polygonum aviculare	Chilbolton	359	366	393	17
	Leckford	381	344	351	17
P. convolvulus	Chilbolton	274	196	163	15
	Leckford	114	159	122	24
	Snoddington	120	145	92	29
Potentilla reptans	Kimpton	903	777	673	64

TABLE 10. *Mean potassium content (% in dry matter) of weed leaves at each site*

	Chilbolton	Kimpton	Leckford	Snoddington	All samples
Brassica sinapis	1·63	1·27	1·82	1·98	1·74
Cirsium arvense	0·84	—	0·47	1·16	0·82
Polygonum aviculare	1·79	1·49	1·58	1·61	1·67
P. convolvulus	1·57	1·11	1·50	1·27	1·44
Potentilla reptans	—	0·58	—	0·84	0·67
Barley (lower leaf blades)	0·64	0·55	1·04	0·90	0·76

Potassium. Table 10 shows the mean potassium content for the various sites and species. The most noticeable difference between species was the low potassium content in thistle and cinquefoil leaves compared with those of the other species, among which black bindweed had slightly lower values than charlock or knotgrass. Where the numbers of samples were sufficient to enable comparisons to be made, site differences were usually significant. Kimpton gave consistently the lowest values of the four sites; this is in agreement with the barley analyses. On the other hand, the comparisons between Snoddington and Chilbolton were inconsistent. In barley, the former showed much higher potassium content values, and the same was true of the charlock samples; in black bindweed, however, a significant difference

in the opposite direction may be noted. Likewise, at Leckford, the potassium content of charlock and barley was significantly higher, that of knotgrass significantly lower, than that at Chilbolton. In none of the weed species (except cinquefoil, for which samples from only two sites were available) did the mean potassium content for the four sites fall into the same order as that given by the barley analyses.

In most instances, an increase in potassium content as a result of application of muriate of potash was evident (Table 11); these increases were, however, not as large proportionately as in the barley samples. The only other treatment effect which reached significance was a reduction in potassium content of charlock leaves at Snoddington as a result of superphosphate application (P_0: 2·18, P_1: 1·85, P_2: 1·92, $F = 4·80$, $P < 0·05$).

TABLE 11. *Effect of muriate of potassium content (% in dry matter) of weed leaves*

		Muriate of potash (cwt./acre)			
Species	Site	0	½	1	S.E.
Brassica sinapis	Chilbolton	1·56	1·74	1·59	0·10
	Leckford	1·76	1·76	1·94	0·10
	Snoddington	1·82	2·05	2·07	0·10
Cirsium arvense	Chilbolton	0·76	0·77	1·00	0·10
Polygonum aviculare	Chilbolton	1·68	1·80	1·88	0·06
	Leckford	1·49	1·54	1·71	0·06*
P. convolvulus	Chilbolton	1·22	1·67	1·82	0·06***
	Leckford	1·25	1·62	1·62	0·14
	Snoddington	0·98	1·44	1·40	0·11***
Potentilla reptans	Kimpton	0·50	0·54	0·70	0·03***

* $P < 0·05$. *** $P < 0·001$.

TABLE 12. *Mean sodium content (% in dry matter) of weed leaves at each site*

	Chilbolton	Kimpton	Leckford	Snoddington	All samples
Brassica sinapis	0·694	0·490	0·461	0·459	0·533
Cirsium arvense	0·694	—	0·727	0·235	0·668
Polygonum aviculare	0·050	0·057	0·050	0·084	0·052
P. convolvulus	0·099	0·072	0·073	0·080	0·084
Potentilla reptans	—	0·121	—	0·063	0·100
Barley (lower leaf blades)	1·175	0·838	0·459	0·761	0·766

Sodium. Table 12 shows the mean sodium content of leaves of the various species at the different sites. The range of variation between species in sodium content is greater than for any of the other elements studied (cf. Collander, 1941), charlock (like other Brassicae), and thistle accumulating sodium almost as much as barley, while the other species had very much lower sodium values. Knotgrass appeared to contain less sodium than black bindweed. Comparing the four sites, the sodium contents of samples from Chilbolton were higher than those of samples from the other sites, both in charlock and black bindweed, as also was previously reported for barley; on the other hand, for knotgrass the means of samples from Chilbolton and Leckford were identical. In cinquefoil, the only species for which such a comparison

is possible, the samples from Kimpton contained more sodium than those from Snoddington.

The only species for which significant treatment effects on the sodium content were found was charlock, in which, as in barley, the sodium content was consistently depressed by applications of muriate of potash (Table 13). The sodium content was also significantly decreased by application of sulphate of ammonia at Chilbolton (N_0: 0·730, N_1: 0·751, N_2: 0·601; $F = 4·50$; $P < 0·05$).

TABLE 13. *Effect of muriate of potash on sodium content (% in dry matter) of charlock leaves*

	Muriate of potash applied (cwt./acre)			
Site	0	½	1	S.E.
Chilbolton	0·821	0·730	0·531	0·038***
Leckford	0·558	0·441	0·386	0·038**
Snoddington	0·578	0·452	0·347	0·038**

** $P < 0·001$. *** $P < 0·001$.

TABLE 14. *Coefficients of correlation between potassium content of barley leaf blades and that of weed leaves from the same plot*

	Between site means		Within sites		Among all samples	
Weed species	*r*	*n*	*r*	*n*	*r*	*n*
Brassica sinapis	+0·695	2	+0·326**	86	+0·385***	89
Cirsium arvense	+0·610	1	+0·156	27	+0·182	29
Polygonum aviculare	−0·497	2	+0·555***	53	+0·224	56
P. convolvulus	−0·258	2	+0·317**	73	+0·201	76
Potentilla reptans	—	0	+0·485**	39	+0·560***	40

** $P < 0·01$. *** $P < 0·001$.

Correlations between potassium content of weed leaves and that of barley. The discrepancies frequently noted in the previous section between site differences in the analyses of barley and of the weed species appeared to render it unlikely that they would show any appreciable correlation. In the case of the element of greatest interest, however—potassium—correlations were computed and tested; they are presented in Table 14. It may be seen that though in four species the correlations between the various plots within each of the sites were highly significant, in no case was this true of correlations between site means. In other words, the potassium content of barley and of the weeds responded similarly to local differences (mainly, it may be supposed, due to the fertilizer treatments) within a soil type, but there was no evidence of similarity in response to differences between soil types.

Correlations between yield response of barley to muriate of potash and potassium content of weed leaves. For each of the plots receiving less than 1 cwt./acre of muriate of potash, an estimate was available of the yield increase which would have resulted from application of ½ cwt. of this fertilizer (see Goodall, 1948*a*). If these estimated yield increases were significantly correlated with the potassium content of weeds

from the same plot, a *prima facie* case would exist that weed analysis could serve for the diagnosis of potassium deficiency with respect to barley. Such correlations were therefore computed (Table 15).

TABLE 15. *Coefficients of correlation between yield response of barley to single application of muriate of potash and potassium content of weed leaves*

	Between site means		Within sites		Among all samples	
Weed species	*r*	*n*	*r*	*n*	*r*	*n*
Brassica sinapis	−0·563	2	−0·236	54	−0·284*	57
Cirsium arvense	—	0	−0·020	18	+0·105	19
Polygonum aviculare	−0·304	2	−0·288	33	−0·074	35
P. convolvulus	−0·846	2	−0·421**	47	−0·394**	50
Potentilla reptans	—	0	−0·069	27	−0·201	28

* $P<0·05$. ** $P<0·01$.

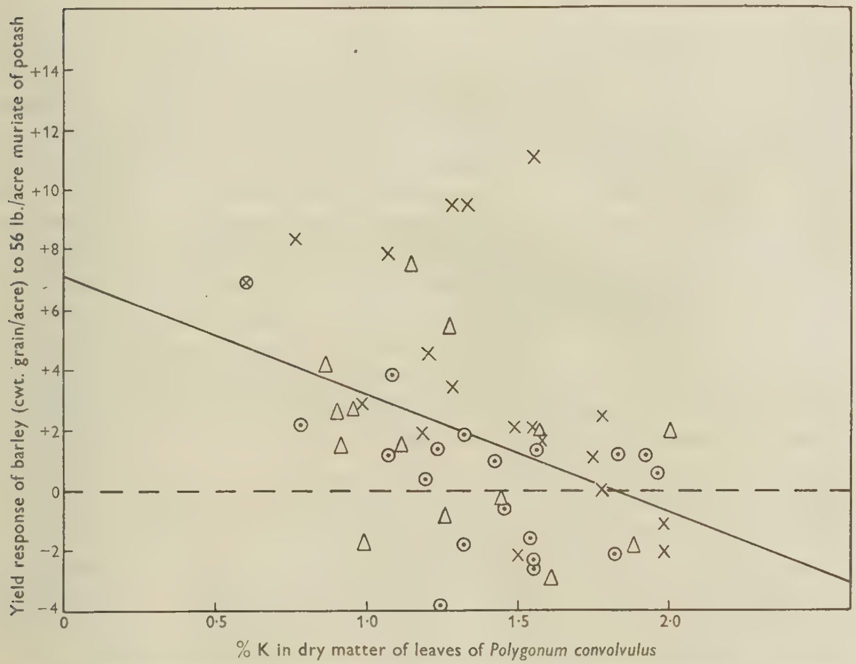

Fig. 1. Correlation between increase in grain yield of barley in response to potassic fertilizer and potassium content of leaves of black bindweed (*Polygonum convolvulus*): × Chilbolton; ⊙ Leckford; ⊗ Kimpton; △ Snoddington.

Noteworthy correlations were observed only in the case of black bindweed. Even here, the correlation as between site means did not reach significance, and, taking the sites separately, only at Chilbolton was the correlation as between plots significant; when the comparisons between plots at each of the sites are combined,

however, the resulting correlation was highly significant, and the same was true if values for all plots were treated together irrespective of their location. The data for this species are presented diagrammatically in Fig. 1, together with the general regression line. It will be noted that this cuts the zero-response ordinate at 1·83%, suggesting that barley grown in localities where the leaves of black bindweed contained more than this amount of potassium would be unlikely to respond to potassic fertilizers; in view of the non-significance of the correlation as between site means, however, this conclusion must be treated with great reserve.

The only other significant correlation is that for charlock, combining data from all plots; since, however, this is an isolated result only just reaching significance, it is probably wiser to ignore it than to base any conclusions upon it.

Discussion

The results described in this paper are unpromising with regard to the purpose which the observations were intended to serve. It appears unlikely that analyses of weeds' leaves would permit reliable forecasts of the response of barley to potassic fertilizers, except perhaps in comparisons confined to a single soil type. A possible exception is black bindweed, but even in this case the value of weed analyses is very dubious. Generally, similar conclusions were reached previously (Goodall, 1948*b*) on the manganese content of weeds in relation to the response of wheat to manganese sulphate sprays, though in those cases where the manganese status of soils from different localities differed very considerably similar differences were observed in manganese content of wheat and of thistle. Such large differences in potassium status did not occur in the four sites included in the present investigation, and the possibility may be admitted that the composition of the weeds might reflect very marked differences in potassium status of soils for barley, just as the differences in manganese status between the soils of central Kent and of Romney Marsh were reflected in the composition of thistle leaves.

The differences between species in the way in which their composition responded to site differences are noteworthy. Apart from differences in the distribution of absorbing roots in the soil and in their ability to absorb nutrients from the various fractions present in the soil, it must be remembered that the course of development of each species through the season and its relation to seasonal changes in the soil will play an important part in determining the final composition of the plants.

It is worth drawing attention to certain consistent differences between species in the proportions between the various cations in their leaf material. Barley, charlock and thistle, for instance, contain very much more sodium than the other three species from the same plots. Barley and the Brassicae have frequently been reported as accumulating sodium, but so far as the writer is aware this tendency has not previously been noted in creeping thistle. Cinquefoil has an exceptionally high manganese content. It may be remembered that Collander (1941) found important

differences in the proportional uptake of cations by different species grown in identical culture solutions.

The author's indebtedness to those responsible for conducting the Hampshire barley trials with which this paper is concerned, and to his colleagues at Imperial College and East Malling for their interest and co-operation, has been mentioned in the paper dealing with the barley analyses, and it is only necessary here to reiterate this acknowledgement. His thanks are also due to Prof. J. S. Turner for reading and criticizing this paper in draft, and to Dr E. S. J. Hatcher for reading the proofs, both of this and the two preceding papers.

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FIELD TRIALS WITH D-D MIXTURE* AGAINST POTATO-ROOT EELWORM

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A D-D mixture has been tested against *Heterodera rostochiensis* at seven 2-acre sites on sands, silts and blackland soils. Apart from a pilot trial where soil was injected in spring, injections were carried out in the autumn, and potatoes were grown the following year. Factors investigated were rate of application (0, 200, 400 and 800 lb. D-D/acre), depth of injection (4 or 8 in.) and the effect of rolling after injection. At the most responsive of the sites (Wainfleet), increases in yield, kill, and the post-crop eelworm population were all roughly proportional to the rate of application. Under favourable circumstances a 50 % increase in yield and something like a 50 % reduction in eelworm population 4 weeks after injection can be expected from 800 lb./acre, but the reduction is more than made good during the growth of the subsequent crop; accelerated multiplication of eelworm on the treated plots leads to their having a larger population than the untreated controls at lifting time. Of the sites tested, the blackland soils gave a lower eelworm kill and a much lower yield increase from D-D than silts or sands. After autumn injection the nematocidal, and probably the phytocidal, effects of D-D persist in the soil for many weeks. The hypochlorite method of 'hatching' eelworm larvae for counting has proved unreliable.

Introduction

Nematocidal effects were recorded by Carter (1943) after using D-D as a soil fumigant in Hawaiian pine-apple plantations infested with *Heterodera marioni* (Cornu). A by-product in the manufacture of allyl plastics, D-D is a black, volatile liquid containing mainly 1-3-dichloropropylene and 1-2-dichoropropane, the former being considered the principal agent toxic to eelworms. Laboratory and small-scale field experiments were carried out at the Institute of Agricultural Parasitology, St Albans, in 1944 and 1945, using D-D against the potato-root eelworm, *H. rostochiensis* Woll. Some of the results, not yet published, were highly promising. Strip injections were carried out in the field using a modified Rototiller, the D-D being gravity fed to small tines behind the Rototiller at a constant rate of about 50 gal./acre and at a constant depth of about 9 in. Comparison of a 12 in. with a 6 in. spacing between the tines gave no significant difference, but a soil covering of Sisalkraft paper improved the results, and, at one site, the rolling of the soil was almost as beneficial as the paper cover.

In January 1946 the Agricultural Research Council (A.R.C.) agreed that more extensive field trials should be carried out by the Institute of Agricultural Parasitology at seven sites in some of the principal potato areas of England, in co-operation with

* 1-3-dichloropropylene, 1-2-dichloropropane, with traces of higher chlorides.

the Advisory Entomologists of those areas, the Shell Refining & Marketing Co., Ltd., and the Statistical Department at Rothamsted.* The senior author organized the experiment on behalf of the A.R.C. and the junior author supervised the laboratory work, at Winches Farm. It is in those capacities that their names appear at the head of this paper. The experiment was, however, a co-operative one, controlled by an *ad hoc* committee convened by the A.R.C.

General considerations

(*a*) *Factors for investigation*

On the basis of previous experience, rates of 0, 100, 200 and 400 lb. D-D/acre were originally planned, but poor results from a pilot trial at 400 lb./acre led to these rates being doubled. It had been found that, at an injection depth of 8–10 in., a large proportion of eelworms in the upper 2 in. of soil escaped destruction by the heavy vapour: injection depths of both 4 and 8 in. were therefore tested, and also the effect of rolling with a Cambridge roller immediately after injection. Since, at the same rate of application, no difference had been found between a 6 and 12 in. spacing of tines the latter spacing was adopted, mainly to avoid impaction with weed residues.

(*b*) *The injector*

The modified Rototiller, drawing two tines, is suitable for small areas but not for the 2-acre plots used in this investigation. The Shell Company made available a tractor-drawn 'metering cart' bearing one row of standard tines and carrying a 50 gal. drum of D-D which was fed to a tube at the back of each tine through a gear pump, one pump to each tine. The pumps were belt-driven from the road wheels and the rate of application of D-D, varied by changing the belt pulleys, was independent of tractor speed within limits.

(*c*) *Experimental design*

The four application rates (including zero), two depths and presence or absence of rolling, yield sixteen different treatment-combinations, two-fold replication giving 32 plots at each site. There are fifteen treatment comparisons and by confounding one component of the triple interaction it is possible to secure four blocks of 8 plots each instead of two blocks of 16 plots. These were arranged as randomized blocks, the two blocks within each replication being separately randomized.

Since the injector covered a strip 4 ft. wide, it was desirable to make the plot width a multiple of this. Plots were, therefore, 12 ft. wide and contained rather more than five rows of potatoes spaced at 27 in. For yield data and soil sampling the two rows nearest the centre of the plot were selected. The plots were 144 ft. long (reduced to 120 ft. in the pilot trial), plus a headland of 30 ft. at each end for

* Detailed acknowledgements are made at the end of the text.

turning the tractor, later increased to 45 ft. Headlands were injected at the highest rate to guard against carrying cysts on to treated plots.

At different sites the four blocks were arranged side by side, 2×2, or tandem, to suit local conditions. The total area at the main sites varied from 1·8 to 2·1 acres, depending on how far the block pattern enabled headlands to be shared.

(*d*) *Criteria of efficacy*

Ware potato tubers and chats from the two rows nearest the centre of each plot were weighed to the nearest pound and the chat weight was recorded as a percentage of the weight of ware plus chats.

Since chemical treatment can lead directly to increased yields without eelworms having been killed, it is desirable to measure nematocidal effects independently. Accordingly, cysts were floated out from air-dried soil samples and a count was made of cysts/g. of soil and of the number of larvae hatching per cyst. The product, larvae/g., was considered the best criterion of the eelworm population. Cysts from a weighed quantity of soil were also added to pots of compost in which tomato seeds were sown; at the 4-leaf stage the seedlings were stained with acid fuchsin and counts made of the stained eelworm larvae within the rootlets to measure the infectivity, rather than the mere viability, of the larvae.

(*e*) *Soil samples*

Soil samples were taken from all the plots on three occasions, these sets of samples being distinguished here as X, Y and Z.

The X samples were taken immediately before injection, to provide an estimate of the pre-treatment population and data for the regression of post- on pre-treatment values by the covariance method.

The Y samples were taken 4 weeks after injection, to measure the immediate effects of treatment. The Z samples were taken immediately after lifting the crop to measure the final effects and to include the new generation of eelworms.

(*f*) *Time of treatment*

To avoid phytotoxic and tainting effects, soil injections were made in the autumn, except in the pilot trial.

(*g*) *Localities*

In the following list the information given is:

(i) Index letter and name for each site.

(ii) National (kilometre) grid reference.

(iii) Rough, one-word description of soil.

(iv) Within quotation marks, a brief description based on soil profiles made by the Soil Survey of England and Wales.

C. *Chatteris*. 52/433866. Blackland. 'Ground-water gley; fine sandy loam, probably former fen peat on gravel.'

G. *Goole*. 44/7718. Warp. 'Warp on peat; ground-water gley. Heavy medium silty loam with sluggish to impeded drainage. Peat lies at 2–3 ft. Carbonate from surface to 24 in.'

I. *Irlam*. 33/7095. Moss. 'The soil is a raised moss peat which has been dressed with town refuse and with heavy dressings of farmyard manure and lime in recent years.'

L. *Littleport*. 52/630910. Blackland. 'Ground-water gley; peaty medium loam on lacustrine, silty clay; slight to fair effervescence from surface.'

S. *Sandy*. 52/193476. Greensand. 'The soil is in the main a freely drained sandy Brown Earth on Greensand with some impedance at the foot of slope.'

W. *Wainfleet*. 53/5158. Silt. 'Ground-water gley, drainage sluggish. Silty light loam on silt, carbonate throughout soil.'

In addition to these six main sites, a pilot trial was run at Gamlingay, the corresponding data being:

P. *Gamlingay*. 52/225528. Greensand. 'Slightly podzolized coarse sandy soil on Greensand. Freely to excessively drained.'

Care was taken to secure level sites so that drainage should not produce confusion by carrying D-D from one plot to another. There was a slight slope only at Sandy; it ran the length of the plots, and the latter were at this site all side by side: there was thus no inter-plot drainage.

(*h*) *Husbandry*

'Majestic' seed was provided from a Northern Ireland source, certified eelworm-free. It was also stipulated that no farmyard manure should be used on the plots. Normal local practice was followed in cultivations and the application of artificial fertilizers.

Methods

(*a*) *Soil sampling*

The normal soil auger proved unsatisfactory on the sandy soil at Gamlingay so corers were used, made from $1\frac{3}{4}$ in. brass or copper tubing. The corer was plunged 9 in. deep at points 12 ft. apart measured along the middle line of the plot, the first point being a random number of feet, between 1 and 12, from the end. Sampling points were successively offset to left and right of the middle line thus: 25 in. L., 5 in. L., 15 in. R., 15 in. L., 5 in. R. and 25 in. R. (repeated), to avoid bias due to row effects. From each plot two bags of six cores each were collected, air dried, mixed, and coarsely sifted to remove stones. Eelworm cysts were floated out from duplicate 200 g. portions of the fine soil by washing it with water as described by Fenwick (1940, Pl. A). Over 1400 bags of soil were dealt with in this way.

(*b*) *Cyst counting*

Floats from the soil-washing apparatus, consisting of cysts and debris, were dried on small squares of bolting silk and stored in tubes. For cyst counting the dried float was transferred bit by bit to Fenwick's counting tray (1940, Fig. 3). For larval hatching cysts were first separated from debris by rolling on sheets of card, and then counted in hundreds on a wet slide.

(*c*) *Counting larvae*

To assess nematocidal effects shortly after fumigation, it is important to differentiate between the living and dead contents of eelworm cysts. Hatching may be stimulated by diffusates from the roots of growing potatoes or tomatoes. If this process is taken to finality, it is fairly safe to assume that all the emerging larvae are living; it has been further assumed that almost all the retained eggs and larvae are dead since, with untreated cysts, very few eggs or larvae were in fact retained. The method is very long and cumbersome and was used only for the crucial *Y* samples.

Alternatively, the contents of crushed cysts may be exposed to calcium hypochlorite, which induces the escape of a proportion of the larvae from their egg shells. By applying both this and the diffusate method to the same samples it was hoped that the proportion chemically released could be related to the proportion biologically hatched. In any event, the hypochlorite method gives some measure of the total number of larvae (living or dead) and, therefore, some estimate of the eelworm population. It was accordingly applied to all the *X* and *Z* samples and, among *Y* samples, to the four main sites (S, C, W, I) which furnished sufficient cysts, and to the pilot site.

A third method, counting stained larvae in the roots of an indicator crop, has been referred to under 'Criteria'.

(i) *Root-diffusate method*

Pots of potatoes under glass were leached about twice a week with water, using about 1·5 times the water capacity of the soil. The excess drainage containing the root-diffusate was stored in a refrigerator, and used only after testing.

Duplicate batches of 100 cysts from a soil sample, after 3 weeks' soaking in water, were treated with root diffusate at 24° C. until hatching ceased, usually about 16 weeks. Weekly, the hatched larvae were removed and preserved in formalin, and fresh diffusate was added. Finally, each tube of preserved larvae was made up to 50 ml. and duplicate 1 ml. aliquots of the stirred liquid pipetted off on to a slide for counting larvae. The total count for each soil sample is in the unit: larvae/8 cysts. In many cases the residual cysts were crushed and their contents counted by the hypochlorite method.

(ii) *Hypochlorite method*

Duplicate batches of 100 cysts were lightly crushed in water and exposed to the action of a calcium hypochlorite solution (5 %, diluted to 1 in 5, which contains less hydrate than a direct 1 % solution), for about 30 min. The suspension was diluted to 50 ml. and duplicate 1 ml. aliquots counted, as in the diffusate method. Some of the larvae 'hatch' from their eggs, and separate counts of eggs and larvae were made.

During the experiment an interesting modification of this technique was devised by Mr P. Bracey. Cysts are taken dry and opened in a wetting agent; after treatment with hypochlorite some eggs become opaque (counted as dead) and others remain transparent (counted as living, whether they hatch or not). This technique gave counts of living larvae in closer agreement with the diffusate counts, but in differentiating dosage levels it was comparable with the standard method, which was retained for the sake of uniformity.

(iii) *Staining method*

Since tomato seedlings would not grow readily in some of the soil samples, cysts were floated out from weighed portions of each sample and intimately mixed with an Innes potting compost, in which seeds were sown. At the 4-leaf stage seedlings were carefully washed free from soil and stained in hot acid fuchsin. Eelworm larvae within the roots are more vividly stained than the root tissues and can be readily counted after clearing.

(*d*) *Statistical analysis*

The paragraphs in this section have been contributed by Mr G. V. Dyke, of the Statistical Department, Rothamsted.

(i) *Sampling errors*

An analysis of individual counts of cysts per 200 g. of soil (one count from each of the four samples/plot) in the pilot experiment showed that the variance of a plot mean was (for four samples/plot) 9407, while for two samples/plot (as in the six main experiments) the variance would be 10,502. The loss in efficiency is thus 10 %. Reduction to one sample per plot would have lowered the efficiency of the estimates by 26 %. The variance between plots (which would be achieved by a complete enumeration of cysts in every plot) was 8312.

(ii) *Analysis*

Yields were analysed directly in the unit: 'lb./two middle rows/plot'. The individual counts of cysts and larvae were summed for each plot and the logarithms of the plot totals used for analysis. The logarithmic transformation was used to make comparable the variances of counts with different means, on the assumption that the standard deviation of a sample count was proportional to the expected value of the count.

The experiment at each centre included all combinations of:

(*a*) D-D applied at 0, 200, 400 and 800 lb./acre.

(*b*) Depth of injection at 4 and 8 in.

(*c*) Rolled and not rolled.

There were two replications and one component of the triple interaction was confounded to give four blocks of 8 plots. The analysis of variance for a normal $4 \times 2 \times 2$ design (Yates, 1937, section 7) was modified slightly to allow for the dummy treatment comparison 4 in. versus 8 in. at zero rate (Yates, 1937, section 15).

(iii) *Covariance*

The analysis of covariance gave no results consistent over the series of experiments. In no case was there a significant regression of yield on initial log cysts/g. or initial log larvae/g. At Wainfleet and Irlam there were similar *positive* regressions of yield on log larvae/g. by the root-diffusate method at Y sampling (each at about the 5 % level of significance), but at the other two sites for which these data are available the regressions were *negative* (but not significant).

The covariance of counts at different samplings were also inconsistent. Only two of the relations examined achieved significance at more than one site and in each case there were both positive and negative regressions of significant magnitude. The coefficients of regression of log larvae/g. (Z samples) on log larvae/g. (X samples) had values ranging from $-0{\cdot}21$ to $+0{\cdot}37$ (none significant). Thus the X counts add nothing to the precision of the treatment effects estimated by final larval counts or by yield.

There was even no consistent correlation between the hypochlorite and root-diffusate counts (in this case measured in the unit 'larvae/cyst'), necessarily carried out on different batches of cysts but from the same soil sample.

Complete data for this experiment are filed at Rothamsted.

The pilot trial

A thorough comparison was made of the Shell injector and the Rototiller injector in a pilot trial. This also tested the field and laboratory methods. The pilot trial, at Gamlingay, was similar in design to the main trials; 32 plots were used (reduced from 144 to 120 ft. long to fit the site), and injection-depths and the effects of rolling were included as factors. The main differences were that the injections were carried out in the spring of 1946 and that, in place of three rates of injection, the following treatments were substituted:

(*a*) Injection by Rototiller.

(*b*) Injection by Shell injector.

(*c*) Injection by Shell injector after cultivation by Rototiller.

The injection rate in all three treatments was about 400 lb./acre of D-D.

(*a*) *Results*

The main comparison between injectors is given in Table 1; all other results will be set out alongside those for the main trials, to facilitate comparison. Table 1 shows the mean yields, and larval counts by each of the three methods, for the four sets of 8 plots each. The larval counts all relate to the *Y* soil samples, taken 4 weeks after injection, and standard errors are not quoted since the listed means are re-transformed from a logarithmic analysis. In this, and all subsequent tables of cyst and larval counts, the values cited are geometric means derived from the re-transformation of the logarithmic values used in the statistical analyses.

TABLE 1. *Pilot trial: comparison of injectors*

Injector	Yield (tons/acre)	Larvae/g. (hypochlorite)	Larvae/g. (root-diffusate)	Larvae/plant (staining)
Control	5·69	100	76	537
Rototiller	8·89	90	45	444
Shell	8·50	57	37	368
Shell, after cultivation	8·17	83	31	462
	±0·330	Geometric means		

Analysis shows that the only significant contrast is that between injected and controls, under each criterion. There is no significant difference between injectors.

The outstanding result in Table 1, however, is the very poor nematocidal effect from D-D at the rate of 400 lb./acre, in spite of injection under almost ideal conditions. There was a good tilth and the soil was clean at injection time and a shower fell just after, helping to seal the soil.

(*b*) *Modifications*

On the basis of experience with the pilot trial the rates of application as originally planned were doubled to 200, 400 and 800 lb./acre. The Shell injector was strengthened and, since the gear pumps failed to give an adequate range of delivery rates, they were replaced by a single piston pump having an adjustable stroke in place of interchangeable pulleys.

The counts of stained larvae in the roots of tomato seedlings were abandoned as they gave results no better than those of the hypochlorite method, at the expense of far more time. The headland width of 30 ft. proved insufficient for turning the tractor and injector, and was increased to 45 ft.

THE MAIN TRIALS

(*a*) *Weather*

Throughout the main trials the exceptional weather seriously affected the experiment, but did not account for the generally disappointing results, as similar results came from the pilot trial under quite ideal conditions.

From June 1946 the weather at all sites was unusually wet; October alone was dry, and all the injections were completed in that month. Soil samples taken at this time showed an abnormally high moisture content for all the black soils. Table 2 shows the mean of the soil temperatures at 4 and 8 in., and the percentage soil moisture. All temperatures were below the 55° F. level recommended by American experience. The low moisture at Sandy reflects the very sharp drainage on this soil.

TABLE 2. *Soil temperature and moisture*

Site	Temperature (° F.)	Moisture (%)
Pilot	45	18
Chatteris	46	40
Goole	51	19
Irlam	46	58
Littleport	53	49
Sandy	53	9
Wainfleet	51	18

Exceptionally heavy rainfall in November greatly complicated the collection, drying and processing of soil samples. The following 4 months were unusually cold with heavy snow; in Northern Ireland frost delayed the opening of the seed potato clamps with the result that seed arrived at the sites very late, at the end of April.

After the snow came the floods. The Littleport site was flooded on 24 March, and remained under water for 8 weeks; when it finally dried it was too late for potatoes. Chatteris was flooded for about a month, but dried in time for planting. Goole was under water for 11 days and was wet for several weeks.

During the growing season, May to September 1947, the weather was abnormally hot and dry, and unsuitable for potatoes. At Chatteris a violent storm on 4 July flooded one corner of the site, seriously affecting crop growth on parts of five plots.

(*b*) *Growth of crops*

(i) *Chatteris.* Early growth was very uneven but by late July it was fair apart from the flooded corner.

(ii) *Goole.* By 31 July all plants were very poor and stunted: the farmer ascribed the failure mainly to spring flooding. Yields were not measured at this site, though the usual *Z* samples were taken.

(iii) *Irlam.* On 1 August there was a good even stand of haulms over the whole site; only some of the control plots could be picked out.

(iv) *Sandy.* On 23 July the plants were thin and poor, about 18 in. high and coming into flower, the control plots appearing slightly poorer than the rest. The potatoes had not been earthed up. By 19 August the potatoes were being smothered by profuse growths of Fat Hen (*Chenopodium album* L.), and in parts the tops were quite dead. The complete crop failure must be ascribed to choking by weeds, partly to drought and no doubt partly to heavy eelworm infestation.

(v) *Wainfleet.* By 19 July the potatoes were in flower and the plots were showing treatment differences. On 30 July the control plots alone were thin and soil showed between the rows; it was not possible, however, to differentiate between the rates of dosage with D-D. The main treatment effect was more clearly visible here than at any other site. This land had not been flooded, and was not badly weed-infested.

(*c*) *Results: dosage factor*

As in the pilot trial, the main experimental effect at all sites was the contrast between injected and control plots with, at some sites, a graduated response at different rates of injection. The other factors, depth of injection and rolling, proved to be of little importance.

(i) *Yields*

Table 3 shows the mean yields by dosage rates at each site and the means for the four sites together. It will be noticed that yields were nowhere very good. The Pilot values are shown for comparison but are excluded from the last column

TABLE 3. *Yields in tons/acre, by dosage rates*

Dose	P	S	C	W	I	Mean
0	(5·69)	0·84	3·61	4·51	7·45	4·10
200	—	1·23	3·77	5·49	7·34	4·46
400	(8·53)	1·17	4·15	6·01	6·62	4·48
800	—	1·18	3·26	6·73	7·74	4·73
S.E.	(0·329)	**0·171**	**0·468**	**0·546**	**0·546**	**0·285***
Mean	(7·82)	1·10	3·70	6·08	7·27	4·44

* Derived from the *treatment* × *site* interaction.

of means. At Sandy the three dosages give approximately equal results 42 % higher than the controls. At Chatteris and Irlam there are no dosage effects. At Wainfleet there is a significant and approximately linear effect of D-D on yield, the yields at the three dosage levels being respectively 21, 33 and 49 % higher than in the controls.

The general mean for percentage of chats was 16·4 % (Sandy, 33·4 %; Chatteris, 8·6 %; Wainfleet, 5·0 %; Irlam, 25·3 %; Pilot, 2·7 %). No significant dosage effects were found and the criterion has not proved useful.

(ii) *Cyst counts*

Table 4 shows the cyst counts from the X and Z samples. There are, of course, no treatment effects in the X samples but they are included to show the range of variation met with under uniform conditions: in so far as these fluctuations are due to topographical variation (rather than to errors of sampling or technique) they may be expected to be reflected in the Z counts. Thus, the final (Means) column of the Z counts suggests that D-D has led to an ultimate increase in the cyst population as compared with the controls, but some doubt is thrown on this when it is observed that, by chance, the same tendency is shown by the X counts. The average increase

in Z compared with X is 44 %, but the increase at 400 lb./acre (37 %) is actually less than the increase in the controls (41 %).

TABLE 4. *Cysts/g. by dosage rates: geometric means*

Sample	Dose	P	S	C	W	I	G	L	Mean
X*	(0)	(2·60)	3·22	1·65	0·63	2·13	0·74	0·79	1·27
	(200)	—	2·94	1·73	0·53	2·33	0·70	1·14	1·31
	(400)	(2·22)	3·22	1·90	0·69	2·18	0·77	1·14	1·42
	(800)	—	3·30	1·85	0·64	2·13	0·69	0·95	1·33
	Mean	(2·52)	3·15	1·77	0·62	2·19	0·72	0·99	1·33
Z	0	(3·18)	4·15	2·81	1·12	3·78	0·95	0·70	1·79
	200	—	4·35	2·94	1·17	4·15	0·91	0·89	1·92
	400	(2·89)	4·35	3·22	1·37	3·70	1·04	0·74	1·95
	800	—	4·25	2·94	1·58	4·35	0·89	0·72	1·95
	Mean	2·96	4·35	3·01	1·28	3·96	0·95	0·76	1·91
	Increase %	(18)	38	70	106	81	32	−23	44

* Treated after sampling.

There is no evidence of a reduced increase in cyst-count due to D-D at any site. Indeed, at Wainfleet there is a just significant *increase*, compared with the controls, which is roughly proportional to dosage rate, and this cannot be explained away by appeal to the X samples.

The last line of the table shows the overall increase due to growing a crop of potatoes, at the five sites where they were grown. This increase is least (32 %) at Goole where the crop failed, and greatest (106 %) at Wainfleet: roughly speaking, the larger the crop of potatoes the larger the crop of new cysts.

No potatoes were grown at Littleport, where the site was under water for 8 weeks, and here there was a 23 % reduction in cyst population. This may have been due to normal decay of cysts or to cysts being floated out by the flood water.

(iii) *Diffusate larval counts*

Geometric mean counts in the unit: larvae/g. are shown by dosage rates for the Y samples from each site, in Table 5.

This technique has given the clearest apparent evidence of a nematocidal effect. Excepting Littleport at 200 lb./acre, counts at all dosage rates are lower than in the controls, at all sites. The final column of means shows a roughly linear response with dosage rate, the percentage reductions being as follows: 200 lb./acre, 17 %; 400 lb./acre, 30 %; and 800 lb./acre, 44 %. Unfortunately, since this criterion is compounded from two others, 'cysts/g.' and 'larvae/cyst', it has high variability, and differences which look suggestive are often not significant.

The effect of dosage rate on root-diffusate Y-counts can be summarized as follows. At Chatteris the effect of D-D in reducing the larval count is not significant, though the counts are suggestive. At Sandy, Wainfleet and Irlam there is a significant and roughly linear response. At Goole the comparison of treated and control is also

significant. At Littleport the linear effect is significant in spite of the anomalous high value for 200 lb./acre.

TABLE 5. *Larvae/g. by dosage rates: geometric means*

Root-diffusate counts

Sample	Dose	P	S	C	W	I	G	L	Mean
Y	0	(81)	143	207	47	184	42	146	108
	200	—	111	193	29	157	32	172	90
	400	(41)	84	197	33	150	21	114	76
	800	—	90	184	22	64	24	84	60
	Mean	(54)	104	193	32	127	29	124	82

(iv) *Hypochlorite larval counts*

This method completely failed to differentiate living from dead larvae. At no site was there a significant nematocidal effect in the *Y* samples, in logarithmic counts of larvae/g., and no useful purpose would be served in quoting the data here. The same is true of the *Z* samples except that, at Wainfleet, there was a roughly linear response which approached significance; the geometric means for the four dosage rates were: 17, 25, 32, 35 larvae/g., suggesting more larvae from the treated plots than from the controls. This point will be referred to later.

At this stage, the only useful information to be gleaned from the hypochlorite counts is the total of eggs and larvae in the three sets of samples for each site as a whole (neglecting dosages) (see Table 6).

TABLE 6. *Eggs and larvae/g. by sites: geometric means*

Hypochlorite counts

Sample	S	C	W	I	G	L
X	87	184	36	174	47	112
Y	72	182	43	213	—	—
Z	181	258	59	436	47	—
Z/X:	2·08	1·41	1·49	2·50	1·00	—

It is doubtful if the hypochlorite method gives reliable counts even of the total population. The count of larvae hatched by root diffusate in the Sandy *Y* samples, 104 (Table 5), is actually higher than the corresponding hypochlorite count of eggs *and* larvae, 72. Thus the tabulated values can be taken as only a rough indication of the total population in the *X* and *Z* samples. The ratio *Z/X* will, similarly, give only a rough estimate of the final increase in population. Nevertheless, there can be little doubt that the increase was greatest at Irlam, where the crop was heaviest and least at Goole, where the crop failed.

Another point of interest in Table 6 is the order of sites in degree of infestation as given by the *X* counts. This order is very different from that of the cyst counts (Table 4), and agrees well with that of the 'untreated' root-diffusate counts (Table 5). Bare cyst counts give a poor indication of the degree of infestation.

(*d*) *Results: other factors*

The results, so far, have been discussed with reference to the factor 'dosage-rate of D-D' only. The other two factors: 'depth of injection' (4 or 8 in.) and 'cultivation' (rolled or not) remain to be considered.

(i) *Depth of injection*

Some criteria at some sites favour 4 in. and others 8 in. depths. With one exception none of these effects is significant. The exception is the criterion 'larvae/g.' by the hypochlorite method applied to the Y samples at Wainfleet, where 8 in. gave a significantly lower density of larvae than 4 in. The average effect over all sites is also slightly in favour of 8 in. depth in respect of yields, Y root-diffusate counts, Z hypochlorite counts, and Z cyst counts. There is therefore slight and inconclusive evidence for preferring a depth of 8 in.

(ii) *Cultivation*

Rolled plots were significantly better than unrolled at Wainfleet in respect of yields, Y root-diffusate counts, and Y hypochlorite counts, and were slightly (but not significantly) better in respect of Z counts of both cysts and larvae. There can be little doubt that rolling was favourable at Wainfleet. The only other significant effects were at Goole, where also the Y root-diffusate counts were lower on the rolled plots, and at Chatteris, where the Y hypochlorite counts were lower on the unrolled plots; Chatteris also slightly favoured unrolled plots in the other criteria. Elsewhere, slight and contradictory effects were shown.

Interactions between the three factors were uninformative, and not significant at any site by any criterion.

(*e*) *Average results over four sites*

Since complete data were available for all criteria from the four sites: Sandy, Chatteris, Wainfleet and Irlam, the more interesting averages are summarized in Table 7, in each case expressed as a percentage of the Control values (this applies equally to the ratios).

TABLE 7. *Averages for four sites* (S, C, W, I): *percentage of controls*

Apart from yields, means are geometric

Sample	Criterion	Technique	Dosage (lb./acre) 200	400	800
—	Yield	—	109	109	115
Y	Larvae/g.	Diffusate	78	75	55
Y	Larvae/g.	Hypochlorite	101	98	93
Z	Cysts/g.	—	106	110	116
Z	Larvae/g.	Hypochlorite	115	118	131
Z/X	Cysts/g.	—	109	104	111
Z/Y	Larvae/g.	Hypochlorite	114	120	141

Of the seven distributions summarized, only the Y larval counts by the diffusate method and the Z/Y larval ratios are fully significant, though significance is approached (particularly in regard to the linear component) in the Z larval counts. The remainder are merely suggestive.

Owing to the absence of a yield response at Chatteris and Irlam, the average yield increases are slight and not significant. In any case, a 15 % increase at 800 lb./acre of D-D would be of no economic importance in relation to the low absolute yields at these sites.

The Y larval counts by diffusate show a maximum reduction in population of 45 % or, neglecting differences between the three levels, an average reduction of 30 %. Even when four sites are compounded, the hypochlorite counts fail to reflect this. Insensitive as this method is, it reveals a roughly linear response which is almost significant in the Z samples and fully so in the ratio Z/Y; the average larval increase on the controls was 82 %, and on the treated plots as a whole 125 %. This is reflected in the Z cyst counts and, unevenly, in the Z/X cyst ratios.

Taking all the data into account, it appears certain that D-D has reduced the eelworm population (as measured 4 weeks after injection) by something like 30 % on the average, the reduction being very roughly proportional to dosage rate. But after the growth of a potato crop, the population on the treated plots has more than recovered from this set-back. All plots show an increase in population by the end of the experiment, but the increase is slightly higher on the treated plots.

(*f*) *Results by soil type*

If yield increases and eelworm reductions (in the Y samples) are examined site by site, it will be found that the sites fall naturally into two groups: the blackland soils where both responses are weaker, and the silts and sands in which they are stronger. This is brought out clearly in Table 8.

TABLE 8. *Yield increase and eelworm kill by sites*

Site		Mean dosage Yield increase (%)		Kill (%)	
C	Blackland soils	3·3	−0·9*	7·6	18·4*
I		−3·0		32·8	
L		—		15·6	
S	Silts and sands	41·6	35·7*	33·6	35·9*
W		34·6		40·5	
G		—		38·8	
Mean			11·2		23·7
Pilot		50·0		49·4	

* Weighted.

The six sites are in two groups of three blackland soils and three others. The former have given negligible yield increases and small eelworm reductions; in this

regard Irlam is something of an anomaly, but Table 7 shows that both here and at Littleport the response is considerable only at 800 lb./acre. This suggests that the black soils require higher rates of application, for comparable effects, than the others. The silts (W, G) and sands (S) give reasonable yield increases and kills as compared with the mean for the six sites. The Pilot values, not included in the mean, are from a greensand soil.

OTHER D-D EFFECTS

Early reports on the use of D-D indicated that it was a highly volatile mixture which quickly left the soil. American reports speak of planting a few days after injection, with no ill effects on the plants. There are data from the present experiment which have a bearing on this matter, and they fall under the three heads: (*a*) residual nematocidal effects, (*b*) taint in tubers grown in injected soil, and (*c*) residual phytotoxic effects.

(*a*) *Residual nematocidal effects*

After this experiment had been started it was noted that American reports claimed, as an advantage of autumn over spring injection, that D-D continued its nematocidal action in the soil during the winter months. Samples taken at Sandy in May 1947 gave root-diffusate larval counts ($Y2$) which are compared with the original Y counts in Table 9.

The Littleport site was sampled at the same time as the other Z samples, autumn 1947. No potato crop had been grown at this site, owing to floods, and this set of samples was comparable with Sandy $Y2$ rather than with Z. The differences are that the interval after injection was 13 months instead of 7, and that the land had been under water for 8 weeks. The root-diffusate larval counts for Littleport are also given in Table 9.

In both cases actual mean counts of larvae/g. are quoted for each dosage as well as being expressed as percentages of the controls.

TABLE 9. *Additional samples* ($Y2$) *at Sandy and Littleport*

Larvae/g.: root diffusate method

		Sandy			Littleport		
Sample	Dose	Actual	% of controls		Actual	% of controls	
Y	0	143		100	146		100
	200	111	78		172	118	
	400	84	59	66	114	78	81
	800	90	63		84	58	
$Y2$	0	176		100	30		100
	200	119	68		35	117	
	400	77	44	47	21	70	76
	800	60	34		20	60	

Taking first Sandy, it is clear that a larger proportion of eelworm larvae has been killed in the $Y2$ samples; that this is a D-D effect is shown by the fact that the

controls have not decreased. In the $Y2$ samples even the hypochlorite technique showed a significant kill, and the diffusate counts showed the 4 in. depth significantly better than the 8 in. Averaging the three dosage rates, it can be said that the kill is 50 % better in the $Y2$ samples (53 as against 34 %).

At Littleport, on the other hand, the results are entirely different: the actual counts show a reduction in the $Y2$ samples to one-fifth of their former value, but this reduction is remarkably constant on all plots, and it can only be assumed that the reduction is mainly due to flooding. The reduction in the final cyst counts was 11 % for the control plots, and 30 % for the injected plots, as against 80 % for larvae. This suggests (*a*) that the main effect of flooding is to drown the larvae rather than to float off cysts, and (*b*) that cysts exposed to D-D disintegrate more rapidly than is usual.

It is difficult to explain why there was no sign at Littleport of the relative residual effect found at Sandy, except that the percentage kill (root-diffusate counts) was here well below the average for all six sites (16 % as against 28 %) and was lowest of all but Chatteris. From the absorbent nature of peat, an enhanced residual effect might have been expected.

(*b*) *Taint in tubers*

From the earlier work in St Albans it was known that tomatoes, grown in soil previously injected with D-D, developed a pronounced and unpleasant taint in the fruit. To decide whether the same applied to potato tubers, a tasting experiment was carried out with the tubers lifted from the pilot trial at Gamlingay. The soil was injected at 400 lb./acre in March 1946; potatoes were set 5 weeks later and lifted at the end of July. Duplicate bags of potatoes, marked *A* and *B*, were issued to nine households for separate boiling and tasting, with the information that one of the two lots came from treated soil. In all, twenty-eight persons assisted in tasting.

All found the control potatoes palatable; twelve found the treated also palatable, but sixteen (57 %) complained of taint in varying degrees: some could not endure more than a single mouthful. The bulk of tainted potatoes, considered unmarketable, was fed to cattle over a period of months, with no obvious ill effects.

The experiment was repeated with potatoes from Sandy, where the soil was injected in October; potatoes were planted in May, and lifted in September. No taint could be detected.

Drs Page and Lubatti of the Imperial College were sent tubers from a pot experiment in which a dose of D-D had been used corresponding to 1460 lb./acre. They could find no trace of D-D by a wet aeration method, and no additional total chlorine in a water extract of pulped potatoes, but the total chlorine of incinerated pulp was significantly higher in the potatoes from injected soil.

It would thus appear that, when potatoes are planted within a month of injecting D-D at 400 lb./acre, a taint may be produced in the tubers which is objectionable to a fair proportion of persons. This taint has a chemical correlate in the form of some

unknown, fixed chlorine compound. At some interval, from 1 to 7 months, between injecting and planting, the taint ceases to be detectable.

(*c*) *Phytotoxic effects*

It has long been known that, at high concentrations, the vapour of D-D is toxic to plants, and it has been found necessary to avoid injecting soil near standing crops, and to allow an interval between injection and planting. This interval varies from a few days to a few weeks, depending on local conditions—especially temperature. The present experiment yielded evidence of phytotoxic effects some 6 (winter) months after injection.

The treated plots and headlands in the Pilot trial were injected at 400 lb./acre in March 1946. After the trial, the entire experimental area was injected at 600 lb./acre in the following October and left undisturbed over winter.

In June 1947, runner beans, marrows, and a few rows of potatoes growing on the site looked unthrifty. An area of very poor growth could be sharply differentiated from the moderately poor by a straight line which bore no relation to the layout of the previous experiment. The line marked the limit reached by spring ploughing when rain intervened for several days. The whole area was planted one day after ploughing was completed, and the worst effect was on the part where only one day separated ploughing and planting.

This is in line with experience in the U.S.A. where, after autumn injections, thorough aeration of the soil in spring is advised. The Gamlingay site was inspected again after a further 5 weeks. The crops had made a complete recovery over the whole site.

Discussion

As judged by the results of this experiment, the immediate prospects for controlling *Heterodera rostochiensis* on a field scale by injecting D-D are not promising. An economic yield increase at the cost of finally increasing the eelworm population cannot easily be defended. At Wainfleet, which has given the clearest response to D-D injection of any site, yield, kill, and final eelworm population all increase in proportion to rate of application within the range tested. In other words, the higher the dose, the higher the yield, the better the kill, and the *higher* the final eelworm population. It is computed that, at Wainfleet, the eelworm population on the control plots increased by a factor of $1\frac{1}{4}$ between the Y and Z samples; on the plots injected at 800 lb./acre the factor was something like $5\frac{3}{4}$, or $4\frac{1}{2}$ times as large. Obviously this rate of multiplication cannot continue indefinitely to be proportional to dosage; there must be some high critical dosage at which the final population is the same as the original, and any higher dosage must lead to a final decrease in the population. The present dosage range has not reached that critical value.

The failure of D-D mixture in England, compared with its success in Long Island, is probably mainly explicable in terms of climate: Long Island is south of the latitude of Rome and also has a continental type of climate. The anomaly that eelworms were

finally more numerous on the treated plots receives some elucidation in the recent paper by Chitwood & Feldmesser (1948) which shows that there is normally a very high mortality of hatched larvae in the soil, of the order of 90 %, due to lack of available space on the roots. A fumigant killing much less than this proportion will merely facilitate the early establishment of the plant, after which the operative law is: the better the plant, the more the cysts. Confirmation comes from Thorne & Jensen (1946, 1947) who reported complete failure of sugar beet from *Heterodera schachtii* Schm. on land which had given a good crop the previous year in response to D-D injection. Probably this is not merely an initial nematocidal effect, since Peters (1948) has demonstrated improved growth of potatoes in eelworm-free soil due to D-D: a partial sterilization effect.

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A COMBINED HAND- OR POWER-OPERATED SPRAYER FOR FLY AND MOSQUITO CONTROL

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(With 2 Text-figures)

A new sprayer is described which was designed in 1945 for the control of adult flies and mosquitoes with insecticides having a kerosene base. This sprayer has been shown in laboratory tests against houseflies, *Musca domestica* L., and yellow fever mosquitoes, *Aëdes aegypti* L., to be highly efficient for both kill and knock-down and to achieve a particularly rapid knock-down of mosquitoes.

The sprayer can be operated with a hand pump or connected to a supply of compressed air. Air consumption is very low and when the sprayer is power-operated, optimum biological efficiency is obtained at 20 lb./sq.in. air pressure. There is no spitting or dribbling from the nozzle and the mist does not settle so rapidly as to cause any appreciable staining of the floor. Although robust it is light, easily manipulated and can be dismantled into component parts for cleaning. It has no adjustments; but the nozzle, which must be machined with precision, is automatically set in its most effective spraying position when screwed home tightly.

Introduction

During the war the control of disease-carrying insects was a problem of major importance. Although some equipment was available to the Services for the application of insecticides, few items had been designed for use in the specialized field of medical entomology. The control methods for which apparatus was needed fell into four main classes:

(1) Large-scale treatment of jungles, swamps, refuse tips, etc.

(2) Application of residual insecticidal films to building and other surfaces.

(3) Local spraying for the immediate control of flying insects.

(4) Treatment of individuals and their clothing.

The insecticide Applications Committee, Ministry of Production, acting on behalf of the Director of Hygiene, War Office, decided that no single piece of apparatus would perform efficiently more than one of these services and delegated to the Pest Infestation Laboratory, which was investigating a wide range of spraying equipment, the responsibility for the development of a hand-sprayer suitable for the local control of flying insects by means of insecticidal mists having a kerosene base and capable of being mass produced.

Existing equipment

In 1943 and 1944 some twenty hand-sprayers, representative of commercial types and of patterns used by various Authorities, were examined to assess their suitability for use with insecticides having a kerosene base. It was thought that among these various types there might be one or more which, after minor modification, would meet the immediate requirements of the Services. The majority of the sprayers were found to be of too flimsy construction and could not be dismantled into component parts for cleaning or for replacement. Generally the nozzle was in an exposed position, its construction crude and its design such that uniformity in performance could not be expected. Little regard had been shown for the physical effort required to operate the sprayers: some caused severe operational fatigue after use for only 2 or 3 min. One type showed promise, and a trial batch of twelve was obtained from the manufacturers. Two of the batch were discarded because they would not produce a spray cloud and the remainder were tested against houseflies by a technique specially devised at the Pest Infestation Laboratory for biological assessment of the performance of hand-sprayers. The range of kill between the sprayers under standardized conditions of test was from 12·7 to 74·5%. It was therefore apparent that no hand-sprayer in commercial production in this country at that time was likely to be able to meet the exacting demands of the Services for robustness and insecticidal efficiency.

Requirements for suitable sprayer

From the work carried out on the commercial types of hand-sprayers and from the experience gained in other work on sprayers at Pest Infestation Laboratory, the chief requirements for a suitable sprayer were formulated as follows:

(1) The spray produced to give a satisfactory percentage kill both of flies and mosquitoes in biological tests.

(2) The construction to be sturdy and the design simple.

(3) The sprayer to be light in weight and the pumping action not to cause undue operational fatigue.

(4) The spray produced to have a carry of at least 4 ft. in still air.

(5) The nozzle to be free from the faults of spitting or dribbling.

(6) The parts of the sprayer and in particular the washers, valves, nozzle, container and dip tube, to be accessible for cleaning or replacement; the nozzle orifice to be protected from accidental damage and adapted for cleaning.

(7) The nozzle to be manufactured with precision to ensure uniform efficiency.

(8) The washers to be made of oil-resistant rubber in preference to leather which, in contact with kerosene, tends to harden and crack.

(9) The sprayer to be adaptable for use by either hand- or power-operation.

Development

The ideal approach to the problem would have been to relate the design to the nozzle to a study of the physical properties of mists shown by biological tests to be effective. The requirements of the Services were, however, too urgent to permit long-term projects. It was decided, in the interests of speed and economy, that component parts of sprayers already in production should be utilized whenever possible and that designs necessitating major alterations in existing manufacturing processes should, so far as possible, be avoided.

At this time two sprayers, one hand- and one power-operated, were in use in this and other experimental work at the Laboratory as standards for comparison of performance. The hand-sprayer, which was of the mixing nozzle type, was selected as the most efficient of the commercial sprayers tested, as judged by physical performance and checked by biological assessment of the toxicity of the spray cloud produced, and its efficiency was increased by small *ad hoc* modifications. The power-sprayer was of the gravity-feed, paint-spray type adjusted to consume a kerosene-based insecticide at 110 ml./min. and air at 4 cu.ft./min. at a pressure of 30 lb./sq.in. In their respective spheres of operation, these sprayers were the most effective tested against flies at the Laboratory, but for various reasons such as flimsy construction, high air consumption rates, and the need for constant maintenance and careful adjustment, they were not suitable for improvement to meet the requirements of the Services. It was, however, agreed by various Service representatives and interested scientists, that the standard at which to aim was the production of a dual-purpose sprayer within the specification already listed and with a biological efficiency equal to that of the standard sprayers in use at Pest Infestation Laboratory.

A point requiring special consideration was the type of spray nozzle to be used. Broadly speaking the types suitable for hand-sprayers could be divided into four groups:

(1) The *opposed jet* in which the liquid is atomized by air delivered at right angles to the liquid jet. This nozzle can give good atomization but the setting of the jets is critical and the air consumption rate is high.

(2) The *swirling jet* in which liquid only is forced through a swirling device and out through the nozzle orifice. The air consumption rate is low but the throw of spray is poor and the spray coarse.

(3) The *mixing nozzle* in which the air and liquid pass into a mixing chamber and are then forced through the nozzle orifice. The atomization can be very good and the air consumption is moderate.

(4) The *mixing and swirling nozzle* in which the liquid and air are mixed before reaching the swirling plate. Atomization is good but the swirling plate reduces the throw of spray and tends to cause dribbling from the nozzle.

After some preliminary work it was decided to concentrate on the 'mixing

nozzle', this being a simple design which allows for wide variation of spray character because the proportions of air and liquid which are mixed in the nozzle can be controlled by adjustment of the size of the passages in the gun body. An experimental sprayer consisting of a body, liquid container, liquid jet and air cap was prepared at the Laboratory. The body had two air passages, one to conduct air into the top of the liquid container and the other leading from the container to the nozzle. This arrangement meant that air used for atomization at the nozzle was first utilized to create a positive pressure above the liquid which was forced up the dip tube and through a passage leading to the nozzle. The Aeraspray Manufacturing Co., Ltd., who were already helping the Laboratory in the development of other types of spraying equipment, were approached regarding problems of manufacture. They supplied various component parts which, without major modification, were suitable for more detailed experimentation and, at the same time, placed at our disposal expert advice on production. Several types of liquid jets and air caps with apertures of different sizes were made and tried in various combinations. The efficiency of each combination was judged visually by the spray cloud produced, the amount of spitting and dribbling etc., and by the effort required to work the sprayer. One combination proved so promising that it was decided to develop it on an *ad hoc* basis, supplementing the observations on physical performance with biological tests of the insecticidal efficiency of those mists judged otherwise satisfactory.

It was found that some measurements could be fixed arbitrarily, but that others, by reason of their influence on the character and effectiveness of the spray mist, could only be decided on after detailed mechanical and biological tests. The diameter of the air passage leading from the liquid container to the nozzle influenced the density of the mist; reduction of this diameter resulted in increased air pressure above the liquid and a correspondingly increased proportion of liquid to air at the nozzle. The length and diameter of the passage in the air cap affected the width of the cone of spray and the incidence of dribbling from the nozzle face. A small increase in the length of the passage very greatly increased the operational fatigue when the gun was hand-operated. The most critical dimension was the clearance between the liquid jet and the internal face of the air cap. Experiments showed that variation of this distance could be related to changes in the toxicity of the spray cloud.

The design (Fig. 1) finally adopted in July 1945 (Higgins, 1945) consists of:

A handle (*A*) cast in aluminium alloy and machined at one end with the standard cone and thread to take $\frac{1}{4}$ in. bore air hose and fitted at the other end with a pricker (7) for clearing the air cap orifice and the liquid jet.

A body (*B*) also aluminium alloy, having two air passages (1*a*, 1*b*), a dip tube (2) with wire-gauze filter, and a liquid passage (3).

A liquid container (*C*) of steel which screws into the body by means of a rolled thread. Between the body and the container is a washer, the only non-metal

component which can come in contact with insecticide; it is essential that this washer should be of oil-resistant material.

A nozzle assembly (*D*) consisting of a stainless steel liquid jet (4) and a brass air cap (6) faced so as to effect a metal-to-metal seal with the body and to ensure the correct clearance between the liquid jet and the air cap. In order to protect the air cap orifice and the thin diaphragm in which it is drilled, the front of the air cap is recessed so that only a direct blow from a small or pointed object can cause damage. Fig. 2 illustrates, in detail, the construction of the nozzle.

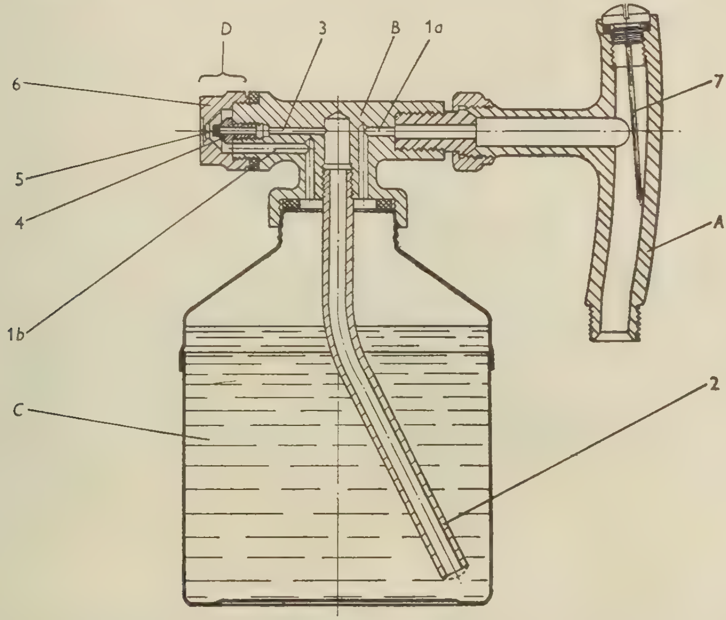

Fig. 1. The new sprayer assembled for power-operation: longitudinal section.

For manual operation a brass pump, having a pump stroke $10\frac{1}{4}$ in. and bore $1\frac{3}{8}$ in. and a non-return ball valve, was obtained from the Aeraspray Manufacturing Co., Ltd. When compressed air is used the pump is unscrewed from the sprayer unit and replaced by the pump handle which then becomes a combined handle and compressed air lead. Air passes from the pump, or through the handle, along passage 1 *a* into the space above the liquid and then to the nozzle via passage 1 *b*. This creates a positive pressure above the liquid which is forced up the dip tube 2 and along passage 3 to the liquid jet. Aided by a slot in the liquid jet the air and liquid are mixed in chamber 5 and the mixture forced through the passage in the air cap.

Twelve sprayers of this final design were manufactured by the Aeraspray Manufacturing Co., Ltd., in September 1945. Three of these (nos. 7, 8 and 9) were arbitrarily selected for detailed biological tests. All measurements and diagrams are of sprayer no. 9 and, although tolerances have not been determined experimentally, it is known that the following variations of the more critical distances can be permitted without danger of adversely affecting the efficiency of the spray mist:

(1) Length of the passage in the air cap	0·024–0·026 in.
(2) Diameter of the passage in the air cap	0·041–0·043 in.
(3) Clearance between the liquid jet and the air cap	0·082–0·084 in.

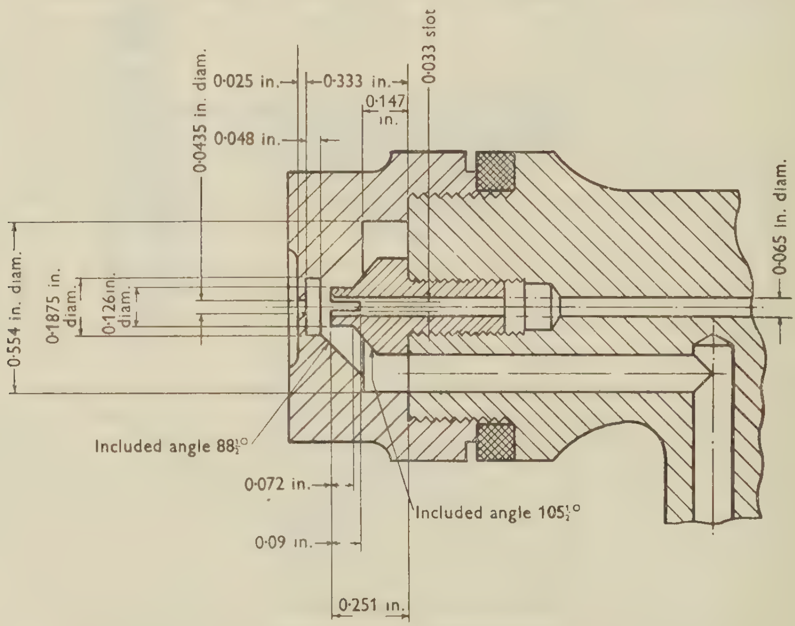

Fig. 2. Nozzle assembly of the new sprayer: longitudinal section.

Mechanical performance of the new sprayer

The sprayer was subjected to a series of tests for general performance. These tests and the results are summarized below.

General information

The weight of the sprayer and capacity of the container are as follows:

Weight of sprayer

Hand-operated, empty	977 g. (2 lb. 2½ oz.)
Hand-operated, charged with 650 ml. kerosene	1526 g. (3 lb. 6 oz.)
Power-operated, empty	497 g. (1 lb. 1½ oz.)
Power-operated, charged with 650 ml. kerosene	1046 g. (2 lb. 5 oz.)

Capacity of container

Full, 804 ml. (1·45 pints).

In practice it should be charged with no more than 650 ml. (1·14 pints).

Optimum biological efficiency was obtained when pumped at the rate of 55 strokes/min. or when connected to compressed air at 20 lb./sq.in.

The sprayer was shown to be completely free of leaks, when subjected to 40 lb./sq.in. air pressure while immersed in water.

Character of spray

(1) *Continuity of spray.* The emergence of spray mist was continuous at pumping rates higher than 44 strokes/min.

(2) *Atomization.* When operated at 55 strokes/min. or by compressed air at 20 lb./sq.in. the sprayer delivered a moderately fine, uniform mist.

(3) *Falling mist.* A sheet of white paper was spread on the floor and the sprayer, while clamped in a horizontal position 3 ft. from the floor, was operated for 2 min. at 20 lb./sq.in. or for 100 pump strokes at 55 strokes/min. The degree of staining caused by falling droplets of spray was negligible by both methods.

(4) *Spitting.* The ejection of an intermittent jet of liquid at the beginning or end of a pump stroke is a common fault among hand sprayers. Tests of the new sprayer under a variety of conditions showed it to be free from this defect.

(5) *Dripping from the nozzle.* Tests showed that with the nozzle design adopted there was no accumulation of spray liquid on the nozzle face and no dripping.

(6) *Throw of spray.* The sprayer was operated at 20 lb./sq.in. when clamped in a horizontal position and, in still air, the effective, i.e. non-turbulent, throw along the centre line of the cone of spray was determined as 6 ft. 6 in. The total forward drift of fine spray in still air was about 16 ft.

Air and liquid consumption rates

The sprayer, connected to a compressed air supply, was clamped in a horizontal position, charged with 500 ml. of kerosene and allowed to spray for 3 min. The rate of air consumption was measured on an orifice-type high pressure air-flowmeter designed by the National Physical Laboratory. The liquid remaining in the container was measured and the rate of liquid consumption determined. Five sprayings were made at each of three air pressures and a summary of results is given in Table 1.

TABLE 1. *Mean liquid and air consumption rates of the sprayer when power-operated*

Air pressure (lb./sq.in.)	Consumption rate	
	Liquid (ml./min.)	Air (cu.ft./min.)
10	50·14	0·43
15	65·70	0·54
20	73·74	0·64

When the sprayer was manually operated the liquid consumption rate for 100 pump strokes was determined. The mean figure for five tests was 95 ml./100 pump strokes.

Biological performance of the new sprayer

Large numbers of houseflies were already being reared for other lines of work at the Laboratory and considerable information had been obtained on the performance of existing apparatus used for control of these insects. Flies were therefore used as test insects for all developmental work but final tests were made on mosquitoes also.

Insecticides

The insecticide used in tests with flies was 0·1 % w./v. total pyrethrins in Pool burning oil (P.b.o.) and was prepared from a concentrate containing 4·80% w./v. pyrethrins. Pool burning oil, the British war-time grade of kerosene, is commonly used as the carrier in fly and mosquito sprays used by the Forces.

In the tests against mosquitoes 'Anti-Mosquito Spray', an insecticide prepared commercially to War Office specification 'to contain not less than 0·03 % pyrethrins and 0·3 % D.D.T.' was used.

Insects

The houseflies, *Musca domestica* L., were reared on an artificial diet at 27·5° C. and 60 % R.H. The adults were fed on a 1:1 mixture of milk and water and were 4–6 days old when used. The mosquitoes, *Aëdes aegypti* L., were supplied as pupae by Ministry of Supply, Chemical Defence Experimental Station, where they had been reared at 28° C. The adults were fed on 20 % sugar solution, were kept at 27·5° C. and 60 % R.H., and used for test when 1–2 days old.

Experimental technique

For the control of flying insects a sprayer should be capable of building up quickly a mist of such concentration and particle size distribution as to give a rapid knock-down and yet deposit a lethal dose of insecticide on the insect. All experiments were therefore designed to give data on both mortality and rate of knock-down.

Tests were made in a chamber of 1000 cu.ft. capacity, measuring approximately 12 ft. × 10 ft. 6 in. × 8 ft. Walls and ceilings were of painted fibre board, held by wooden battens; the concrete floor was covered with clean white paper during experiments.

Test insects (300–400) were released into the chamber, the temperature of which was maintained at 24–27° C. About 5 min. later, the operator stood in the centre of the floor holding the sprayer at shoulder height, i.e. 3 ft. from the ceiling, pointing it towards the angle between ceiling and walls, i.e. at an angle of about 30°, and slowly turned while spraying. To finish the spraying the operator backed towards one wall so as to spray towards the ceiling. The dose of insecticide used was 35 ml./1000 cu.ft. for the fly tests and 10 ml./1000 cu.ft. for the mosquito tests.

Estimations of the percentage of insects knocked down were made at 5, 10 and 30 min. from the start of spraying, after which an exhaust fan removed any remaining mist. In the fly tests four lots of fifty flies, and in the mosquito tests all the mosquitoes, knocked down on the floor paper were then collected, placed in muslin cages and stored at 27·5° C. and 60 % R.H. for mortality counts. The flies were given diluted milk and the mosquitoes sugar solution as food. Counts of dead flies were made 24 hr. later but, in all except the first test, the mosquitoes were left for 48 hr. before counting. As only the female mosquito is the vector of disease, mortality counts for the two sexes were recorded separately. Insects remaining in the spray chamber were counted and destroyed. The walls of the chamber were swabbed with a 1 : 9 mixture of acetone and industrial methylated spirit. After drying, the fumes were removed by the exhaust fan, and the floor recovered with clean white paper.

One of the two, previously mentioned, standard sprayers, of a known high level of efficiency was included in each test for purposes of comparison.

In the course of the development and final testing of the new sprayer, twenty-eight spraying experiments were carried out, involving the use of approximately 50,000 flies and 10,000 mosquitoes. It is, however, proposed to describe only tests on the final design.

Results of biological tests

Three sprayers were tested against houseflies both as hand- and as power-operated sprayers. Results are shown in Tables 2 and 3.

TABLE 2. *Biological tests against flies: results from three new sprayers when hand-operated*

Dosage 35 ml./1000 cu.ft.

		Toxicity of spray mist to flies			
		Estimated knock-down (%)			Kill % in
Exp. no.	Sprayer	5 min.	10 min.	30 min.	24 hr.
FS 165	No. 7	98–100	100	100	61·0
	No. 8	98–100	98–100	100	66·5
	No. 9	98–100	100	100	60·5
	Standard hand-sprayer	95–98	98–100	100	68·0
FS 169	No. 7	98–100	100	100	74·0
	No. 8	98–100	100	100	74·5
	No. 9	98–100	98–100	100	77·5
	Standard hand-sprayer	98–100	100	100	74·0

Controls, not sprayed: FS 165 (424 flies) 11·1 % dead in 24 hr.
FS 169 (411 flies) 1·2 % dead in 24 hr.

The new sprayers were shown to be similar in biological performance to the standard sprayers, which had been selected for their high efficiency. Having shown that the new sprayer was satisfactory for killing flies it was necessary to test its

efficiency against adult mosquitoes. Limited numbers of these insects were available and it was therefore not possible to test all three of the sprayers which had been used in the fly tests. No. 9, which, as already stated, is the actual sprayer from which all

Table 3. *Biological tests against flies: results from three new sprayers when power-operated*

Dosage 35 ml./1000 cu.ft.

		Toxicity of spray mist to flies			
		Estimated knock-down (%)			Kill % in
Exp. no.	Sprayer	5 min.	10 min.	30 min.	24 hr.
FS 168	No. 7	98–100	100	100	72·0
	No. 8	98–100	100	100	73·5
	No. 9	98–100	100	100	81·5
	Standard power-sprayer	95–98	98–100	100	71·0
FS 168*a*	No. 7	98–100	98–100	100	77·5
	No. 8	98–100	100	100	82·0
	No. 9	98–100	98–100	100	84·5
	Standard power-sprayer	98–100	100	100	81·0

Controls, not sprayed: (431 flies) 5·3 % dead in 24 hr. (both experiments carried out on same day)

Table 4. *Results of tests using the new sprayer against adult mosquitos*

Dosage 10 ml./1000 cu.ft

				Mortality counts			
		Estimated knock-down %	Time for 100 % knock-down	Males		Females	
Exp. no.	Sprayer	(5 min.)	(min.)	Total no.	% dead	Total no.	% dead
FS 171	New sprayer hand-operated	100	4	138	94·2	186	86·0
	Standard hand-sprayer	80–90	17	218	97·2	190	88·4
	Controls, not sprayed	—	—	197	2·0	207	1·5
FS 176	New sprayer hand-operated	98–100	6	155	98·1	145	94·5
	Standard hand-sprayer	85–90	9	194	99·0	163	97·6
	Controls, not sprayed	—	—	198	1·0	172	2·9
FS 177	New sprayer hand-operated	100	5	231	99·2	187	97·9
	Standard hand-sprayer	90–95	8	236	99·6	283	97·8
	Controls, not sprayed	—	—	280	2·5	270	2·6
FS 178	New sprayer hand-operated	100	4	256	99·6	86	100
	New sprayer power-operated	100	6	126	100	42	100
	Standard hand-sprayer	85–90	9	358	97·2	85	94·1
	Controls, not sprayed	—	—	309	1·0	74	1·4
FS 179	New sprayer power-operated	100	5	264	100	242	98·8
	Standard hand-sprayer	85–90	8	217	98·2	191	96·9
	Controls, not sprayed	—	—	270	1·8	260	3·7

measurements are quoted, was selected for test against adult mosquitoes. Five experiments were carried out; results are shown in Table 4. In FS 171 mortality counts were made 24 hr. after spraying, but it was found that up to 12 % of the

treated insects, although badly affected, were not dead: in subsequent tests counts were made at 48 hr., when almost all insects could be classified as either normal or dead. The results recorded for Exps. FS 178 and FS 179 represent only a part of the data from larger experiments in which other sprayers were also tested. It was necessary for these larger experiments to include the standard hand-sprayer, but unfortunately there were insufficient numbers of insects to complete the comparisons by including the standard power-sprayer.

These experiments showed that, under the conditions of test, the new sprayer gave a high kill of adult mosquitoes and was quite as effective as the standard hand-sprayer. It was equally effective whether hand- or power-operated. The outstanding result of these tests was the high rate of knock-down of mosquitoes achieved when using the new sprayer.

We are indebted to the Aeraspray Manufacturing Co., Ltd., and in particular to Mr F. B. P. Rambridge of that Company, for much helpful advice during the development of the Sprayer, and to Chemical Defence Experimental Establishment, Porton, for supplying mosquitoes for the tests. The drawings were prepared by the Metrology Division, National Physical Laboratory.

This work has been carried out as part of the programme of research of the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research.

REFERENCE

Higgins, A. E. H. (1945). The design and use of sprayers for insect control. *Brit. med. Bull.* **3**, 229.

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A VERTICAL SPRAYING APPARATUS FOR THE LABORATORY EVALUATION OF ALL TYPES OF LIQUID PEST CONTROL MATERIALS

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(With Plate 7 and 5 Text-figures)

Following a discussion of methods for the uniform application of insecticides for evaluation purposes, a description of a vertical spraying apparatus is given. The spraying procedure is described. By varying different factors and estimating the consequent variation in the replication of deposit the accuracy of the apparatus was determined. The main advantage of the present method lies in the possibility of accurate and quick readjustment of the nozzle by means of a calibrated adjusting nut and a fixed vertical pointer. The nozzle is constructed of stainless metals and is not easily damaged.

Introduction

For the accurate evaluation of insecticides it is necessary to use a spraying apparatus providing a high degree of reproducibility over a wide range of dosages. A review of the literature showed that a vertical spraying apparatus of the type originally described by Tattersfield (1934) presented the best possibilities, although it was not accurate enough for our investigations. The area that could be covered evenly with a spray deposit was too small and it proved difficult to readjust the nozzle to its former position after cleaning.

During the war the Royal Dutch Shell Laboratory, Amsterdam, modified and developed the Tattersfield vertical spraying tower to the apparatus that will be described below. After the war, when the literature published in the allied countries during 1940–5 became available, it was found that Potter (1941) had also improved the Tattersfield apparatus, working along slightly different lines. Our apparatus, therefore, is not an improvement of Potter's spraying tower, but a parallel development of the Tattersfield apparatus, having certain new features.

To enlarge the area covered evenly with a spray deposit Potter tried a great variety of atomizing nozzles. He came to the conclusion that none of them was satisfactory. All had the disadvantage of lacking a means of adjusting vertically the position of the tip of the liquid jet in relation to the tip of the air jet, by which adjustment the degree of atomization is markedly influenced. Accordingly, Potter designed and constructed an atomizing nozzle in which the liquid jet could be moved up and down in the air jacket and centred by means of three adjusting screws. Hewlett (1946) improved the nozzle considerably. During the war one of us (Kraak)

developed a nozzle with which accurate readjustment to a former position was quickly and easily possible.

This nozzle was made in the Shell Laboratory workshop. Data on its performance in practice are given. The first spraying tower was made in 1942, and has been in constant use ever since. Various improvements have been made resulting in the apparatus now described. Several nozzles have been made since and are now in use in other laboratories.

Description of apparatus

Atomizing nozzle

The nozzle (Text-fig. 1) is constructed of two separate parts: an inner liquid feed tube (*a*) and an outer air jacket (*b*). The latter is connected by a tube (*c*) to a supply of compressed air. The liquid feed tube (*a*) is turned off concentric with the air jacket in such a way that the interchangeable tip (*d*) when moved up or down remains concentric with the air jet (*e*). The liquid feed tube consists of stainless steel 18–8 and is 15 cm. long so as to ensure accuracy in centring.

Another special feature of this nozzle is that it has interchangeable monel metal tips with various diameters (0·45, 0·7 and 1·0 mm.) which makes it possible to spray homogeneous liquids of widely differing viscosities, e.g. water, kerosene, spindle oils, as well as emulsions and suspensions. The construction of one of these metal tips is shown in Text-fig. 2. The concavity at the end of the tip is provided to ensure even coverage of the sprayed area. It prevents the effluent spray liquid from moving to one side should the nozzle axis not be exactly vertical. Special reservoirs were also developed to provide the most satisfactory container for each of the spray types mentioned.

A fixed vertical pointer (*f*) is mounted on the air jacket opposite a calibrated adjusting nut (*g*). Thus any nozzle setting can be reproduced quickly and accurately. The calibrated adjusting nut and the liquid feed tube are one piece.

The tangential entering air jacket (*b*) has an air supply tube, constructed in such a way that, when entering the air jacket, the air receives a rotating movement. This promotes the even distribution of deposit on the sprayed area. The cone tip of the air jacket is made of stainless steel, the other parts of brass.

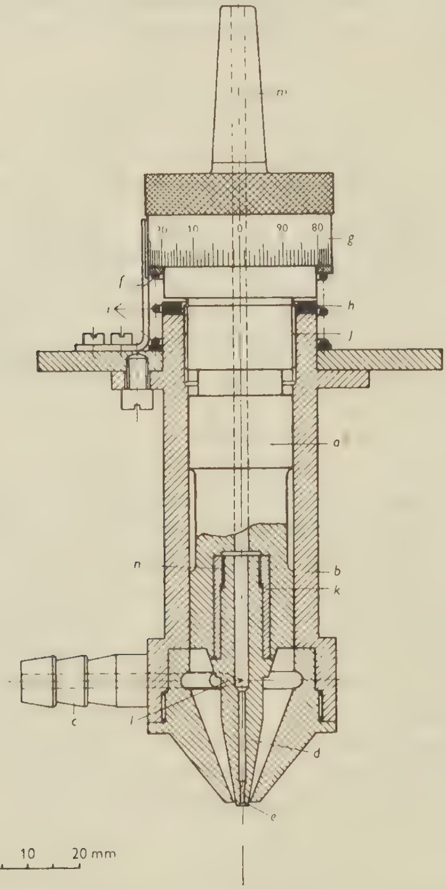
Spray chamber

The spray chamber (Pl. 7) consists of a plexiglass cylinder (*B*). This type of plastic proved more suitable than cellon, as the latter material easily absorbs dyestuff and then becomes less transparent. The total height of the cylinder is 60 cm., the internal diameter 30 cm., the gap between the lower flange of the cylinder and the splash trap (*C*) 5 cm.

Splash trap

A splash trap consisting of a brass plate (*C*) is provided to collect the excess spray that does not reach the object table and to support the plexiglass cylinder (*B*).

Through a 12 cm. hole (C') the spray jet passes to the object table (G). A rim around the hole in the splash trap prevents soiling of the object table perpendicularly under the nozzle opening. Spray collecting on the plate is removed through two narrow tubes in the bottom.

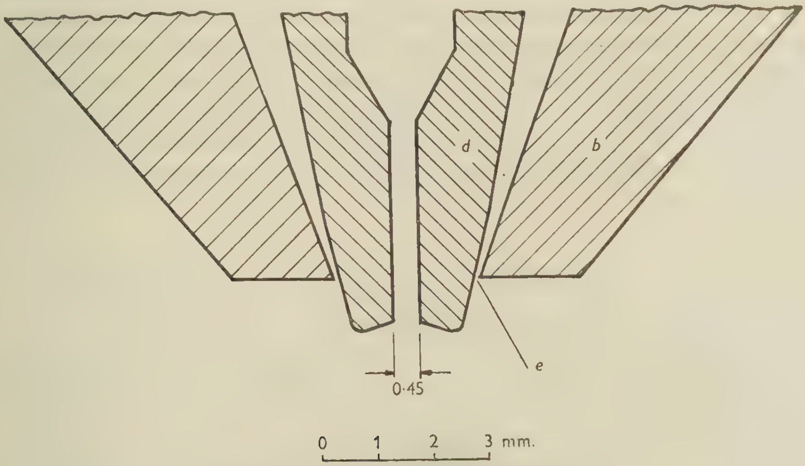


Text-fig. 1. Details of nozzle. *a*, liquid feed tube; *b*, air jacket; *c*, air tube; *d*, exchangeable tip; *e*, air jet; *f*, fixed scale; *g*, adjusting nut; *h*, ring to prevent damage to tip; *i*, spring; *j*, threads of liquid feed tube; *k*, gland; *l*, tangential air tube; *m*, conically ground joint; *n*, threads of exchangeable tip.

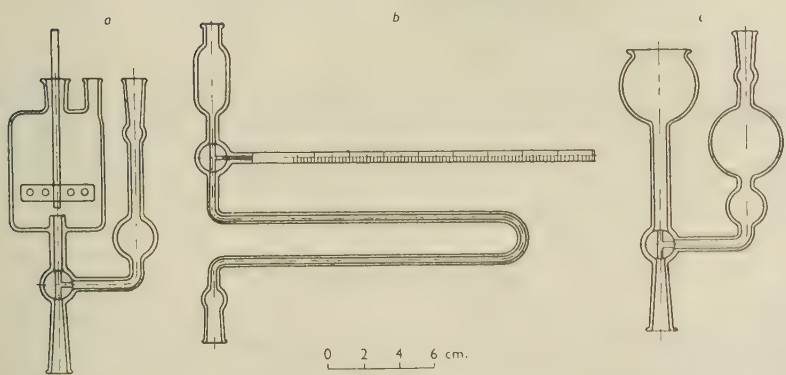
As the whole spraying apparatus is placed in a fume cupboard provided with an exhaust and the sprayed object is removed immediately after spraying, the excess spray mist issuing from between the bottom of the cylinder and the splash trap does not present any difficulties.

Masking disk (*F*)

To prevent the initial spray from reaching the object a removable plexiglass masking disk is turned over the hole in the brass plate (*C*). This disk is not turned aside until the whole spray tower is filled with a homogeneous spray cloud. The liquid collecting on the disk can be run off by a slight tilt.



Text-fig. 2. Tip of the spraying nozzle.



Text-fig. 3. Types of spray reservoirs: (*a*) for suspensions; (*b*) for straight oils; (*c*) for solutions in kerosene etc.

Rotary disks (*D*)

Two aluminium disks of 38 cm. diameter and connected with each other to rotate simultaneously, are driven by a synchronous motor (*H*). The upper disk has a grooved edge to accommodate the driving belt. Both disks have two diametrically

opposed open sectors with an angle of 77° through which the spray can reach the object. By moving one disk with respect to the other it is possible to diminish the total sector width. The extent to which the total sector is blocked can be seen from the calibrated upper disk.

The object table (G)

The brass object table has a diameter of 11 cm. and can rotate about a shaft. The table can be adjusted to any height and serves to place the object to be sprayed quickly and accurately in position under the spray nozzle and to remove it after spraying. Its surface area of 100 cm.^{2} can be covered evenly with spray deposit. The position of the insect on this area is therefore of no importance. Filter-paper or cellophane disks can be clamped to the object table by means of a tightly fitting brass ring.

Spray reservoir (L)

Various types of spray reservoir are used, dependent on the nature of the spray liquid (Text-fig. 3 models (*a*), (*b*) and (*c*)).

Model (*a*) is used for *suspensions*, which have to be stirred continuously, and the volume sprayed is measured from readings on the side tube. The same model, but without a side tube, is used for emulsions, as with these materials the spray dose is applied by means of the rotating disk (see p. 399).

Model (*b*) is used for *straight oils*, which are generally measured by volume, as the slow rate of flow of these more viscous liquids allows of accurate reading.

Model (*c*) is used for low-viscosity *solutions* in kerosene, etc.

Compressed air supply to the nozzle

Compressed air derived from a compressed air line or cylinder passes the following devices to ensure an even and constant flow of clean air through the nozzle: an air receiver to equalize flow (not visible on the photograph), a reducing valve (not visible on the photograph), a pressure regulator (*I*), a thermometer (*O*), an air flowmeter (*M*) and an air filter (*N*).

Spraying technique

Methods

In our spraying tests the amount of toxicant was varied either by using a range of dosages, at one concentration, or by using various concentrations generally in one prefixed dosage. The first method has the advantage that, by making use of the rotating disk with open sectors, spraying can be done very quickly, as the spray liquid need not be changed after every test. The number of revolutions at a given speed are used as a measure for the amount of spray applied. This method has some drawbacks, however. At high dosages losses may occur due to running off of the liquid on to the absorbing filter-paper underneath the insects. With very low dosages,

on the other hand, it is possible that vital parts of the insects are not covered by the widely scattered droplets.

In most cases, except, for instance, when using straight oils, the second method is preferable, being more related to the spraying procedure in actual practice. As the spray liquid must be changed after every concentration, this method is more time-consuming.

Estimation of the dosage by weighing the spray deposit

To estimate the amount of spray that will reach the object, filter-paper disks are clamped to the object table if dosages higher than 1 mg./cm.^{2} are given, while cellophane disks are used with dosages of less than 1 mg./cm.^{2}, the hygroscopic nature of the filter-paper giving rise to inaccuracies. With higher dosages the liquid would run off from cellophane, whereas filter-paper can absorb this greater amount of liquid. The amount of spray liquid reaching the surface of the 100 cm.^{2} disks is determined by weighing the disks before and after spraying.

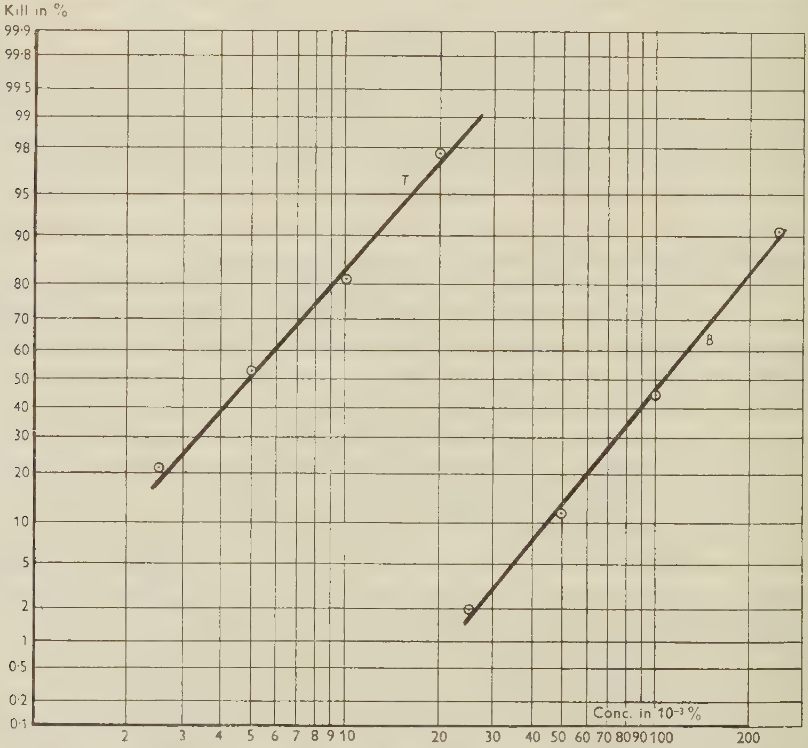
In the first method the rotating disk has to make as many revolutions as may be necessary at the given speed of rotation to get the dosage required. This number of revolutions should be a multiple of that for the lowest dosage that is sprayed. By enlarging or narrowing the sector opening the initial dosage can be adjusted.

Using the second method the rotating disk is allowed to make one revolution, then the deposit is weighed and from this weight the sector width and/or the speed of rotation to get the dosage required is calculated. After adjustment, the deposit with this new sector setting and/or changed speed is measured.

In adjusting the dosage required before spraying the object all deposit estimations are made in three replicates. The deposit is afterwards rechecked in two blank sprayings. For more viscous liquids (oils), which require a longer spraying time, it is not practical to use the rotating disk. For these liquids the time of spraying or the volume that is sprayed to get the deposit required is used as a measure. As a rule, both volume and spraying time are used, the one being a check on the other.

When using the rotating disk the spraying procedure is as follows: The spray liquid (e.g. an oil emulsion) is put in a reservoir (of the type shown in Text-fig. 3*a*) and stirred continuously. The object is put on the object table underneath the rotating disk, the centre being vertically under the nozzle. The plexiglass masking disk is closed, so as to prevent the spray jet from reaching the object. The air supply is adjusted to 18 l./min., as shown by the air flowmeter (*M*). By opening the valve of the reservoir above the nozzle the spray is obtained. After the spray cloud has filled the outer cylinder the masking disk is turned away at the moment the closed part of the rotating disk is covering the object. After the required number of revolutions (sector openings) the masking disk is replaced at the moment when the closed part of the rotating disk is again above the object. Then the valve of the spray reservoir is closed; the object having thus been sprayed with a known dosage of the insecticide.

When spraying on a time or volume basis the rotating disk is fixed in such a way that the sector opening (fully opened) is just above the object table, the masking disk covering the object. The spray jet again fills the cylinder for a few seconds, after which the masking disk is turned aside, a stop-watch being started simultaneously. At the end of the spraying time required the disk is quickly put in the closed position again. If the spraying time used is not too short, say > 8 sec., the



Text-fig. 4. Comparison of the mortalities obtained with solutions of Toxaphene (*T*) in aromatic-free kerosene and γ-isomer of benzene hexachloride (*B*) in the same solvent on houseflies (*Musca domestica*). In view of the high mobility of the flies, spraying was measured by volume (stationary disk) at a dosage of 0·66 mg./sq.cm. The flies (2 × 150 per test) were sprayed on their backs in specially designed rings, covered at the top by very thin steel wire with interspaces of 1·5 mm.

time error is insignificant. Simultaneous estimation of the volume sprayed after turning aside the masking disk increases the certainty that the spraying procedure was normal. This estimation is only used with liquids which have a slow rate of outflow. An example of the good results obtainable with this method is given in Text-fig. 4.

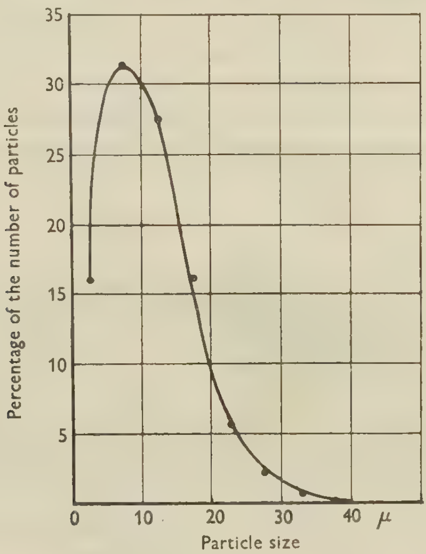
The only time we rely exclusively on volume readings is when spraying rather coarse suspensions giving rise to partial clogging of the nozzle.

Testing the accuracy and adaptability of the apparatus

Distribution of deposit

It is an essential requirement of this type of apparatus that the whole area of the object table (100 $cm.^2$) should be covered with an even spray deposit, irrespective of the particle size that is being employed. A homogeneous spray jet must therefore be produced.

The correct settings of the liquid feed tube in the air jacket to achieve this were obtained as follows: A solution of methylene blue in water was sprayed on to disks of white filter-paper placed on the object table. The evenness of the field distribution was estimated visually when using various nozzle settings. The speed of the air jet



Text-fig. 5. Droplet size distribution of a light mineral oil sprayed with the apparatus described.

was standardized at 18 l./min., giving the rather low pressure of 0·5 atm. Temperature of the air was standardized at 20° C. and the room in which the experiments were carried out was air-conditioned (20° C. and 65–70 % R.H.). Alterations or adjustments in nozzle setting are made until an even coverage with methylene blue solution is obtained. The relative positions of the adjustable nut and the vertical pointer are then recorded. A similar spray pattern can then be obtained on any future occasion by resetting of the nozzle in this position. The distribution and particle sizes of the spray droplets were then estimated by using glass plates coated with Drifilm and spraying straight oil. Microphotographs are taken which are used for the measurements.

The nozzle is evaluated for every exchangeable tip in this way so that a wide variety of conditions can be reproduced at will. Specimen filter-paper disks sprayed with methylene blue solutions and a record of the respective settings of the adjustable nut and vertical pointer are kept for reference purposes. In general it can be said that the most favourable position is reached when the tip of the liquid feed tube extrudes about 1 mm. from the air jacket (Text-fig. 2).

Once the right nozzle setting to obtain an even spray pattern is known, the droplet size distribution can be obtained by measuring the droplet diameters on a photomicrograph of a part of the sprayed surface of a coated glass plate. Text-fig. 5 gives an example of the droplet size distribution when 0·2 mg./cm.^{2} of a combination of 9 parts kerosene + 1 part white oil is sprayed.

Since the size of the insect or its egg is also known, it is possible by this method to estimate the number of droplets of any given size that are likely to fall on it at any given dosage rate. It is probable that in certain cases where an insecticide is used at low dosage rate, the resulting kill may be very low, not because the material is non-toxic, but because it does not reach the eggs in sufficient quantity. When this has been proved, a more effective kill may be obtained by increasing the amount of liquid, so that sufficient material actually reaches the egg.

Reproducibility of deposit

It is essential that any apparatus of this type should give a high degree of reproducibility at any dosage rate. Table 1 gives the results obtained with a 1 % summer oil emulsion applied in a rather high dosage: it was diluted with tap water and constantly stirred. Table 2 shows the results obtained with a straight spindle oil in a low dosage. In each case twenty-five separate determinations were made. The high degree of reproducibility is shown by the fact that the percentage standard deviation was ±2·92 in the former and ±2·72 in the latter case.

TABLE 1. *Replication of deposit in mg. when using a 1 % oil emulsion sprayed on to 100 cm.^{2} of filter-paper*

Room temp. 20° C.; R.H. 65 %; air velocity 18 l./min.

	Test series				
Weighing no.	1	2	3	4	5
1	279	272	278	274	259
2	277	270	281	275	278
3	268	268	260	268	273
4	274	267	280	249	260
5	264	261	265	276	265
Average deposit (mg.)	272	268	273	268	267
Standard deviation	6·0	4·3	9·6	11·0	8·3
Percentage standard deviation	±2·3	±1·6	±3·5	±4·1	±3·1
Overall standard deviation			7·9		
Overall percentage standard deviation			±2·92		

TABLE 2. *Replication of deposit in mg. when using a straight spindle oil*

For the conditions see Table 1

Weighing no.	Test series 1	2	3	4	5
1	29·9	29·5	28·4	28·9	29·5
2	29·3	28·0	27·8	29·2	29·4
3	30·7	28·5	26·2	28·2	28·0
4	31·4	28·9	27·1	27·9	27·7
5	30·0	29·9	28·2	28·7	29·7
Average deposit (mg.)	30·5	29·0	27·6	28·6	28·7
Standard deviation	0·85	0·75	0·93	0·51	0·86
Percentage standard deviation	±2·8	±2·6	±3·4	±1·8	±3·0
Overall standard deviation			0·78		
Overall percentage standard deviation			±2·72		

To ascertain whether this high degree of reproducibility is influenced by the diameter or height of the cylinder, by the use of vertical screens in the cylinder to decrease air turbulence or by a variation in air pressure different tests were made. Table 3 gives part of the results, twenty-five determinations being made per tested item. A cylinder of less than 30 cm. diameter was not tested because it is known that the spray liquid then collects on the sides, thus obstructing the view of the rotating disk. Only a small influence on the percentage standard deviation could be detected when cylinders of various sizes exceeding 30 cm. were used. The lowest standard deviation was invariably found when a cylinder with a diameter of 30 cm. and a height of 60 cm. was used. We therefore chose these dimensions for our standard apparatus.

TABLE 3. *Influence of cylinder shape on percentage standard deviation*

Room temp. 20° C.; R.H. 65 %; dosage adjusted by means of rotating disk; liquid sprayed on 100 $cm.^2$ of filter-paper; spray liquid, a 0·05 % sodium alkyl (C_{10}–C_{18}) sulphate solution in tap water; weighing in mg. (on aperiodic balance); air velocity 18 l./min.; twenty-five observations per tested item

Cylinder	Average deposit (mg.)	Percentage standard deviation
No cylinder	242	7·0
Diameter 30 cm., height 60 cm. normal position (see Pl. 7)	268	4·2
Same cylinder resting on splash trap	318	9·0
Diameter 60 cm., height 60 cm.	241	7·0
Diameter 30 cm., height 30 cm.	286	6·1
Diameter 30 cm., height 60 cm. with radial sheet metal plates up to half the diameter	236	10·5
Same cylinder with radial gauze sheets up to half the diameter	225	6·5
Same cylinder with radial sheet metal plates reaching halfway up over half the diameter	273	5·1
Same cylinder with radial gauze sheets reaching halfway up over half the diameter	263	5·4
Same cylinder but with a fine copper gauze in the cone of the nozzle round the interchangeable tip	191	4·8

The use of vertical gauze screens had no appreciable effect on the variation in replication of deposit. With sheet metal plates a small influence was found, but even then the standard deviation was not more than 11 %. No influence of air velocity on the percentage standard deviation could be detected. The average total deposit was, however, higher when the air velocity was increased.

Our conclusion is that, when in general use by reasonably skilled operators, the above spraying tower will give deposits to within plus or minus 3 % of those actually required when normal aqueous solutions, oil emulsions or straight oils are used, this being 5 % for solutions with extreme surface activity. This accuracy can be obtained at dosage rates as low as 10 mg./100 cm.^{2}.

Discussion

The spraying apparatus and technique described have been used extensively for the following purposes and on the insects mentioned:

(1) Contact insecticidal experiments with solutions, emulsions and suspensions on: housefly, cockroach, various beetles, caterpillars and aphids. Special containers suitable for the different insects were used.

(2) Residual insecticidal experiments with accurate dosages of the insecticides in solvents, emulsions and suspensions on different surfaces.

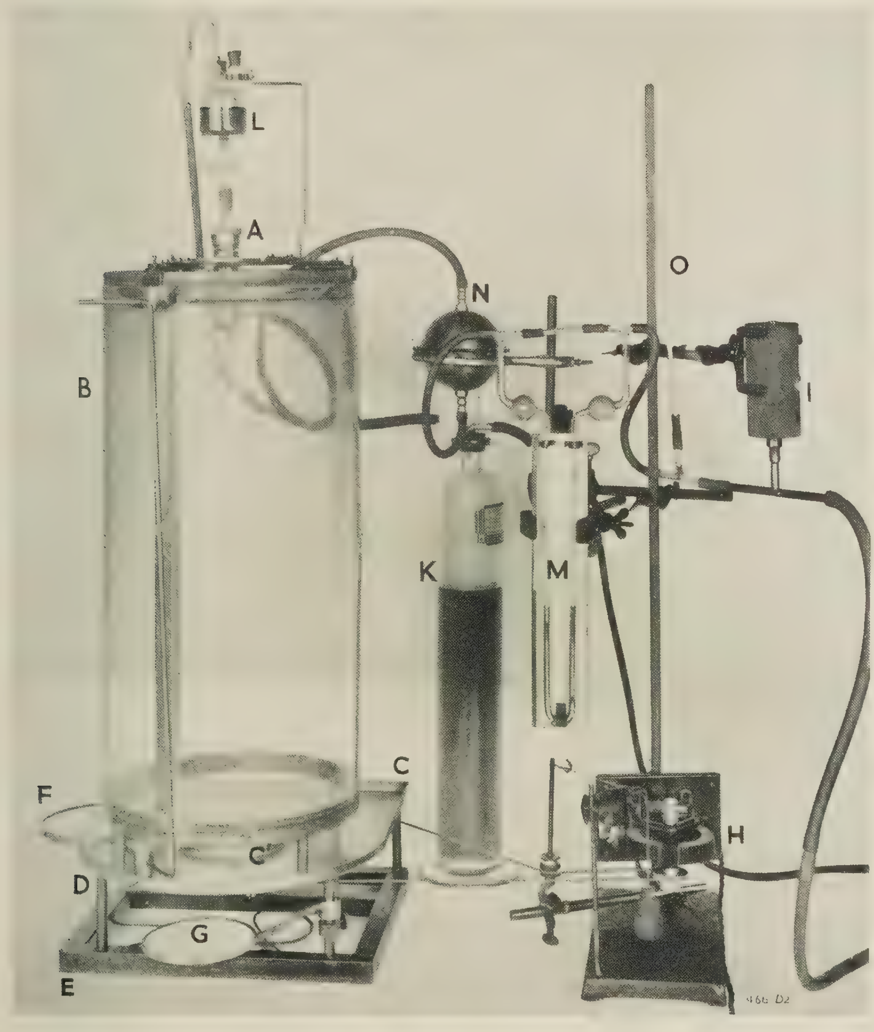
(3) Ovicidal experiments on aphid eggs, red-spider eggs and flour-moth eggs.

(4) Fungicidal experiments, spraying fungicidal liquids on leaves and other surfaces.

(5) Phytocidal experiments on cucumber plants and apple seedlings.

In all experiments the apparatus proved highly satisfactory if slight modifications were made to suit the type of experiment being conducted. The accuracy of the replication of deposit is about the same as with the Potter apparatus (1941) and with the special nozzle developed by Hewlett (1946). The advantages of the nozzle here described are the possibility of accurate and quick readjustment to the proper position and the use of exchangeable tips with different orifices. The greatest accuracy is, of course, required from the technician who makes the nozzle, as there is no way of centring the liquid feed tube in the air jacket during the experiments, as is the case with the Potter and Hewlett nozzles. We therefore chose a rather lengthy nozzle, which is easier to centre by the technician.

We wish to express our thanks to Dr Swarbrick for his valuable advice and criticism and to Mr Hewlett, whose comments on the draft of this paper were thankfully incorporated in our present article. Dr Dierick, Miss Schuytvlot and Mr Heringa, all of our Laboratory, made valuable suggestions during their work with the laboratory spraying tower described and therefore contributed considerably to the construction of the final apparatus.



TEN HOUTEN AND KRAAK—*Vertical spraying apparatus for liquid pest control materials*

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EXPLANATION OF PLATE 7

Vertical spraying apparatus *A*, nozzle; *B*, plexiglass cylinder; *C*, splash trap; *D*, rotary disks; *E*, stand; *F*, masking disk; *G*, object table; *H*, synchronous motor; *I*, pressure regulator; *K*, water manostat; *L*, spray reservoir; *M*, air flowmeter; *N*, air filter; *O*, thermometer. (The apparatus as it is now being used has the air filter *N* *before* the air flowmeter *M*.)

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REVIEWS

The Plant in Health and Disease. By W. A. R. Dillon Weston and R. Eric Taylor. Pp. 173. Agricultural and Horticultural Handbooks. London: Crosby, Lockwood and Son, Ltd. 1948. 21*s*.

This book will be of interest and value to the student, the advisory officer and the farmer or grower. The student will find it a good introduction to the basic principles of what constitutes health and disease in plants, and to the main groups of plant disease and their causative organisms. The advisory officer, and particularly the district officer, who has to be a 'general practitioner in food production' will find that it explains well the methods of practical plant pathology. The farmer and grower will find in it a readable account of many of his own particular troubles, combined with much sound advice.

The account is a good summary of the whole field of Plant Pathology. The chapters deal briefly with ancient and modern views on plant disease, the green plant in relation to its environment, the biology of fungi and spore dispersal, classification of disease organisms, symptomatology, principles of plant protection, modern methods of seed treatment, selected diseases carried by seed and soil, some tomato diseases, diseases spread by airborne spores, nutritional troubles and virus diseases. Finally, there are two useful chapters on legislation and technical advice. An appendix gives a list of common plant diseases and their causes.

In general, the book is notable, as is indicated by Prof. Scott Watson in his foreword, in giving a well-balanced account of the subject and in emphasizing the maintenance of plant health rather than the treatment of plant disease. The illustrations are excellent and many of them are original.

L. OGILVIE

Svalof 1886–1946. *History and Present Problems*. Edited by A. Akerman, O. Tedin and K. Froier; English Technical Editor, R. O. Whyte. Lund: Carl Bloms Boktrycheri A. B. 1948. Price 42*s*.

Just as it is virtually impossible to include in one book of this size the accumulated experience, knowledge and philosophy of plant breeding acquired over 60 years of continuous work, so is it impossible to give an adequate appraisal of the significance of the Svalof contribution to plant breeding in a short review of this book. There are all too few accounts of breeding methods, techniques and achievements, and some books which purport to be plant breeding manuals are little more than accounts of the genetics of the plants with which they deal. This book from Svalof is consequently of the utmost significance and interest to plant breeders and agriculturists, while geneticists might be well repaid for reading it if only to acquire an appreciation of the problems of breeding in distinction to those of genetic investigation.

This book consists of twenty-two separate articles each written by one or two authors, though some authors have written more than one article. The result is not a manual of plant breeding, but a survey of 'the experience, ideas and work' of the Svalof Institute. The articles vary in their scope, content and treatment, there being general descriptions of the Swedish Seed Association and the climate and soils of Sweden, specific accounts of the breeding of the individual crop plants handled at the Institute, and descriptions of special breeding techniques such as the artificial induction of mutations and polyploids.

The organization of Plant breeding in Sweden is a model of its kind and explains to a large extent the success of plant breeding in that country and its considerable contributions to agriculture. Sweden has been ahead of most countries in its appreciation of the potential value of plant breeding in raising the standard of agricultural production, and this has been due to the realization as early as 1886 of the necessity of improving the varieties of crop

plants. Starting as a private undertaking Svalof is now a national necessity, and has developed into a government-sponsored organization financed by Government, Agricultural Societies, the General Seed Company which markets Svalof productions, private donors, laboratories and miscellaneous contributors. The whole organization now includes the breeding, seed propagation and marketing of all the major crops in each of the important areas of production of Sweden.

Among the more technical chapters devoted to breeding techniques, one of the most interesting is that by Akerman and MacKey on the breeding of self-fertilized plants by crossing. This account is especially welcome as being a frank and unbiased description of the various practices in handling hybrid progenies and the advantages and disadvantages of different methods of selection. It is of some significance that the reader must conclude that the breeder must decide which method to use according to his own judgement. There is no golden rule, but 'for successful breeding the breeder has to become familiar with his segregating material', and in spite of the improved techniques, 'it is still the breeder's intuition and his subjective judgement which are of the utmost importance for a successful result to his breeding work'.

It cannot be said that this book contributes anything entirely new to the generally accepted techniques of breeding, or that it helps to overcome the inherent weakness of the choice of the best parents for hybridization, the best methods of selection, the quickest way of judging the value of a cross, etc. There are efficient tools to aid the task of selection in the form of yield trials, quality tests, cold-resistant tests, etc., but the chapters on the breeding of winter and spring wheat, oats and barley indicate that Svalof breeding is tackling its problems by similar methods to those used in this country, though on a larger scale. The Swedes, however, have had the great advantage in their cereal breeding by possessing a great reservoir of breeding material in their 'land varieties' or races.

There is no doubt that the Svalof methods have proved themselves in their contribution to breeding achievements and to scientific research. In the self pollinating cereals the exploitation of straight selection, and of transgressive segregation and utilization of the so-called 'population' as opposed to the 'pedigree' method of selection in handling hybrids have been effective tools in the hands of the breeders as is exemplified by the number of cereal varieties which have been put on the market.

The position is not as satisfactory in certain other crops as it is in cereals. In hybridizing cultivated varieties of potato, for example, 'the choice of parents may be more or less haphazard...it is difficult or even impossible to obtain any kind of system or proof that certain varieties are good parents, and others poor parents, but our experience is that it is almost impossible to predict anything about the outcome of a cross on the basis of the characters of the parents used'. The lack of a fully satisfactory method of breeding in cross-fertilized root crops such as sugar beet and mangolds, is also a matter of comment, although singularly good results are reported from inter-strain hybridization in sugar beet.

Sweden is now particularly interested in Brassica crops as a source of oil, and work has been concentrated on winter and summer rapes (*Brassica napus*). X-ray mutations of value have been obtained in summer rapes, while artificial tetraploids have been produced in most of these oil crops, though those in white mustard appear to be the most promising. This is in contrast to soya beans which have not proved responsive to either of these breeding techniques. In flax and linseed direct line selection from old varietal populations has proved unsuccessful in fibre types, but very successful in oil types, while X-ray chlorophyll mutants with superior characters have resulted from treatment of fibre types although artificial polyploidy has not yielded anything of worth.

The organization of the Svalof investigations is based on a thorough appreciation of the fundamental importance and significance of the part played by ecological concepts in breeding and agriculture. This is seen particularly in the accounts of the breeding of the cross-fertilized herbage plants, where great emphasis is laid on the utilization of the available occurring natural variation, while considerable space is devoted in this book to accounts of

the breeding systems, self-sterility and self-fertility phenomena, etc., in the various species concerned.

The great variation in ecological conditions in Sweden has necessitated the development of a number of branch stations which may engage in local independent breeding, in co-ordinating breeding between more than one station and the headquarters at Svalof, and in local variety testing. They also provide data for phenological and biochemical studies, and collect valuable information on Swedish agriculture. The general significance of this type of investigation is discussed in relation to varietal and strain adaptation in cereals and herbage plants to the light and temperature factors, and an attempt is made to analyse the principal climatic factors which exercise selective influence, while the additional effects of cultivation and the incidence of pests and diseases are considered. These discussions serve to emphasize the distinction between an analytical genetic problem and the broad sweep of the agro-ecological demands of a breeding problem.

The contrast between the above concepts and the cytogenetic work which has been conducted at Svalof shows clearly the development of the diverse techniques which is at present characterizing breeding investigations. The production of abnormalities or monstrosities has little place in agricultural plant breeding, but techniques which will result in the production of useful variations are valuable. A significant part of our present knowledge on the practical breeding value of artificial autopolyploids produced by colchicine, and of X-ray mutants, is the result of Swedish work, and the balanced account of this work given by the Svalof workers is particularly valuable. There is here no uncritical optimism of the direct production of valuable agricultural plants, but rather the realization of the necessity for painstaking and long-term research which does not offer any easy short-cut to plant improvement. Workers in this country who have engaged in distant hybridization and artificial polyploidy as a breeding technique, as the reviewer has, will agree with this attitude, though the advances made by the Swedes in the production of valuable X-ray mutants in such crops as barley and flax are promising of better things in the future in this particular field.

This book, which is very well produced, with the subject-matter clearly presented, will be of interest and value to a wide circle of readers engaged in the study of crops and their improvement. There are many good photographic illustrations and many chapters have a bibliography of relevant literature by the Swedish workers. G. D. H. BELL

Gall Midges of Economic Importance. Vol. IV: Ornamental Plants and Shrubs. By H. F. BARNES. Pp. 1–165. Agricultural and Horticultural Handbooks. London: Crosby, Lockwood and Son, Ltd. 1948. 15*s*.

This is the fourth of eight volumes by Dr Barnes on the gall midges of economic importance. This volume deals with a number of midges of great importance to the nurseryman including gall midges of flowers, and in particular, that very serious pest the chrysanthemum gall midge. To many readers the term 'ornamental plants' is synonymous with 'shrubs' and a better title might have been 'Gall Midges of Flowers and Shrubs'.

The work fully maintains the high standard of the first three volumes. The indexing and references of these books is so good that it is a sheer delight to use them. I. THOMAS

L'Hérédité. Par E. GUYÉNOT. 4th ed., pp. 625. Doin, Paris. 1948.

This work, which first appeared twenty years ago, is part of a set of three on Heredity, Variation and Evolution by the same author. By the standards of this country for a Professor of Zoology to write even one work on genetics (running as it does to 625 pages with 77 pages of bibliography) is surprising enough. Prof. Guyénot, however, began breeding *Drosophila* in 1913 and seems to have kept it up ever since. His point of view is of course largely zoological and he looks at genetics from the point of view of a science teacher to whom the chromosome theory is an academic exercise of boundless interest and an end in itself. That is to say he has not realized the uses it has developed during the last ten years. Structural

changes in chromosomes are the basis of experiments with *Drosophila* in the laboratory rather than of the origin of species in the wide world outside. And his illustrations of chromosomes are of the standard of zoology and botany text-books in this country: they are mostly thirty or forty years old. In a word, Prof. Guyénot is what the Russians would call a reactionary mendelist-morganist. But in spite of all these things he has written a text-book of heredity which many zoology laboratories in this country would find it useful to have.

C. D. DARLINGTON

Carotinoide. Von P. KARRER und E. JUCKER. Pp. 388 (*Lehrbücher und Monographien aus dem Gebiete der exakten Wissenschaften*, 17: Chemische Reihe, Band III). Basel: Verlag Birkhäuser. 1948. 43 Swiss francs.

The carotenoids, a group of pigments responsible for many of the yellow, orange, red and brown colours in nature, are widely distributed in plants and animals; in the latter they are apparently derived from, although not always identical with, the plant carotenoids in the animal's food. They have become increasingly a focus of study; partly, because of their intrinsic chemical interest, especially in relation to molecular structural pattern, and, partly, because of their biological interest in relation to the formation of vitamin A and to the physiology of vision and phototaxis in animals, and, in plants, in relation to flower and fruit coloration and its genetic behaviour, to the functional activity of auxin-*a*, and to fertilization in certain algae and possibly other micro-organisms. During the last two decades, the application of micro-methods of oxidation and reduction and of chromatographic and spectrum analysis has resulted in a swift and detailed elucidation of their chemical structure, whilst knowledge of their natural distribution and functions in the animal and plant body has been enormously extended. These researches have been published in many languages and in numerous and often not easily accessible journals; the authors have co-ordinated the results of this work to the end of 1947, and have set them out in masterly fashion in this volume.

The book is divided into a general and a special part. In the first eight chapters of Part I (pp. 13–70) the authors describe the condition in which carotenoids exist in living things, their origin and their physiological significance, their chemical constitution (illustrated by full structural formulae), their methods of isolation, determination and synthesis, and the relation between their constitution and their colour. In Chapter IX (pp. 71–105) there is set out the detailed distribution of carotenoids throughout the animal and plant kingdoms. The organisms are tabulated in their systematic groupings and arranged alphabetically by genus and species within their Family or Order. The specific carotenoids each organism produces are noted, with reference in each case to a bibliography of 434 citations at the end of the chapter: this bibliography would have been much more convenient if it had been arranged alphabetically by authors. Chapter IX is the outcome of a most thorough searching of the whole range of biological and biochemical literature and it summarizes in easily available form a vast amount of information.

Part II (pp. 113–365) is devoted to a consideration of the special aspects of the sixty-five known carotenoids. It contains five chapters, each restricted to one chemical group of the pigments, and each carotenoid is discussed in a more or less standardized way—historical perspective, tabulated occurrence of the particular carotenoid in organisms which are arranged systematically and with bibliographic reference to footnote citations, details of preparation, physical and chemical characteristics with full structural formulae, and derivatives. Part II ends with two coloured Plates showing the crystal forms of carotenoids, and twenty-eight graphs of absorption spectra. There is a name index to the 1300 or so organisms mentioned in the text, and a general index.

Like the previous issues in this Series the volume is beautifully produced and it is a model of what such a Monograph should be. Although its interest is, perhaps, primarily biochemical, it will be invaluable as a source book and reference text to all biological workers in this important and rapidly advancing field of study.

W. B. BRIERLEY

Mathematical Biophysics. By N. RASHEVSKY. Revised ed. Pp. xxiii + 669. The University of Chicago Press and Cambridge University Press. 1948. 42*s*.

This is the second and greatly enlarged edition of a book originally published in 1938. It incorporates much of the material of Rashevsky's *Advances and Applications of Mathematical Biophysics* (Chicago, 1940) and other research published in the author's journal *The Bulletin of Mathematical Biophysics*. The book does not deal with statistical methods in biology or with the mathematical theory of the balance of animal populations developed by Volterra and others, but rather with those mathematical problems which are suggested by physiology and biophysics. It is divided into four parts. The first contains an extensive discussion of the mathematical theory of the form, stability, growth and energy relations in cells, and whilst most of the discussion is theoretical, experimental results are compared with theory wherever possible.

The second and third parts, occupying some three hundred pages, deal with the theory of the peripheral and central nervous system respectively. Here experimental verification and comparisons are much more difficult and the discussion is almost entirely theoretical. It is noteworthy that no use is made of the interesting physiological and psychological applications of the theory of servomechanisms, a theory which has been greatly developed during the war in connexion with anti-aircraft gunnery, and whose concepts appear to be extremely suggestive in physiology (see N. Wiener, *Cybernetics*, Paris (Hermann)).

The last part deals with the biological organism as a whole and considers form in plants, and form and movement in animals. An interesting chapter deals with the movement of snakes and another with birds and insects, but it is probable that a good deal of work has already been done on these subjects which is not referred to here.

The book as a whole raises the important and difficult question of how far mathematical analysis of this kind is useful in biology. That the subject raises a number of interesting problems for mathematicians is clear, but the fact that much of the work lacks experimental comparison and verification may raise doubts in the minds of many biologists. In the reviewer's opinion this would be somewhat mistaken for often the qualitative deductions from a mathematical theory may be of great importance in laying bare the mechanisms at work which determine both quantitative and qualitative phenomena in biology. To see this one has only to think of the importance of the ideas involved in such other applications of mathematics to biology as Volterra's theory of the balance of animal populations or the work of Fisher and Haldane on the mathematical theory of natural selection, theories in which numerical verification is difficult or impossible but which have, nevertheless, proved of great importance in biological thought.

Most biologists will find the mathematics of this book fairly stiff reading but it is to be hoped that some of those working on the problems with which it deals will read it. Advance in this subject can only be made by collaboration between mathematicians who have sufficient interest and knowledge to talk to biologists about the latter's problems, and biologists who have sufficient elementary acquaintance with mathematical ideas to realize where mathematical investigations may be a help to them. This book, whose printing and binding are admirable, will help both and deserves to be widely read. P. A. P. MORAN

Die Larvenformen der Dipteren. Von W. HENNIG. Pp. 186 with 60 Text-figures and 3 Plates. Berlin: Akademie-Verlag. 1948. DM. 22.

This is the first of three parts of what will certainly be a valuable and important contribution to the literature on Diptera. An introductory or general section of 64 pages examines phylogenetic relationships in the Diptera and the importance of larval and pupal systematics in such studies. The general and comparative morphology of Dipterous larvae is outlined and there are original diagrams depicting evolution from the lowest forms of the Nematocera to the highest of the Brachycera.

The 'special section' begins with a classified list of the families of the Order; the author recognizes only the two sub-orders Nematocera and Brachycera, the former he divides into Bibiomorpha and Culicomorpha and the latter into Tabanomorpha and Muscomorpha. All the Bibiomorpha are dealt with in this volume. Thirteen families including the Bibionidae, Mycetophilidae and Itonididae (Cecidomyiidae) are described. The systematic position of each family is discussed and its larvae and pupae are characterized. Typical life cycles are described and geographical distributions discussed. The keys for both larvae and pupae are generally taken to the genus and they are supplemented by good illustrations. There are very full references to the literature which, however, will be catalogued only at the end of Part III of the work.

Although few families of importance to economic entomologists are dealt with in Part I, the completed work will fill a serious gap in the literature on economic entomology.

I. THOMAS

REPORT OF THE HONORARY TREASURER FOR THE YEAR ENDED 31 DECEMBER 1948

During the year ended 31 December 1948, the amount received for subscriptions and entrance fees, including arrears, was £648. 10s. 0d., which is £74. 0s. 6d. more than in 1947. The continuance of the steady rise of the last ten years is most satisfactory. However, the numbers of members in arrears has increased from fifteen at the end of 1947 to twenty-six at the end of 1948.

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £1472. 18s. 0d., an increase of £199. 9s. 6d. on the year. The cost of production was £2095. 13s. 7d., a decrease of £90. 13s. 11d., although the number of pages was the same as in the previous year. This decrease is accounted for by a decrease in the cost of paper and printing as well as in binding, offset by an increase in carriage, insurance and accommodation. The income from the sale of back volumes and parts was £277. 3s. 6d., an increase of £38. 9s. 1d., while the sale of reprints more than doubled showing an increase of £64. 6s. 9d. The balance against the *Annals* on the year's working decreased from £583. 3s. 7d. to £230. 6s. 4d., an improvement of £352. 17s. 3d.

Income for the year showed an excess of £379. 0s. 4d. over expenditure. This is accounted for by the decreased balance against the *Annals* and the increase in subscriptions and interest. The Association received from the Royal Society a grant of £150 in aid of publication. This gift is gratefully acknowledged.

In view of the future obligations to those members who have compounded under the new rule 5, the life-membership fund reserve has been increased to £150 from £25, the figure at which it previously stood. This is a reasonable sum to cover all those members who have compounded their subscriptions.

It will be noticed from the Balance Sheet that the accumulated interest on the 500 National Savings Certificates which were originally purchased for £390. 1s. 0d. is now £432. 16s. 5d. It can also be seen from the General Income and Expenditure Account that the interest receivable for the year is given as £21. 13s. 0d. Last year it was £25. 8s. 9d. This matter is being taken up with the authorities.

After all obligations had been met, the net assets of the Association at the end of the year were £410. 5s. 4d. more than a year ago, and amounted to £2148. 7s. 2d. All surplus funds available are invested in the Post Office and National Saving Certificates. The estimated value of the stock of the *Annals of Applied Biology* is £117. 5s. 0d.

THE ASSOCIATION OF APPLIED BIOLOGISTS

ANNALS OF APPLIED BIOLOGY, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1948

Dr. / Cr.

Expenditure	£ s. d.	Income	£ s. d.
To Estimated Value of Stock, 1 January 1948	126 18 0	By Sales—Current Volume	1472 18 0
To Cambridge University Press	2095 13 7	By Sales—Back Volumes, Parts and Sets	277 3 6
		By Sales of Reprints and Adverts	124 18 9
		By Estimated Value of Stock, 31 December 1948	117 5 0
		By Balance, carried down	230 6 4
	£2222 11 7		£2222 11 7

GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1948

Dr. / Cr.

Expenditure	£ s. d.
To *Annals of Applied Biology*, balance brought down	230 6 4
To Printing and Stationery	30 15 5
To Postages and Cheque Stamps	26 11 7
To Subscription—Parliamentary Science Committee	7 7 0
To Donation—The Biological Council	5 0 0
To Sundry Out-of-Pocket Expenses of Editors, Secretaries and Treasurer	6 11 7
To Expenses of Plant Pests and Diseases Committee	8 10
To Expenses of Conversazione	38 15 10
To Audit Fee	5 5 0
To Balance, being Excess of Income over Expenditure for the Year	379 0 4
	£730 1 11

Income	£ s. d.	£ s. d.
By Members' Subscriptions:		
Arrears	14 5 6	
Entrance Fees	36 14 6	
Current	597 10 0	
		648 10 0
By Sundry Income (Conversazione)		24 3 0
By Net Receipts—*List of Common Names*, Pt. 1		5 15 7
By Interest Receivable:		
National Savings Certificates	21 13 10	
Post Office Savings Bank Account	29 19 6	
		51 13 4
		£730 1 11

BALANCE SHEET, 31 DECEMBER 1948

Liabilities and Surplus	£ s. d.	£ s. d.
Sundry Creditors:		
Cambridge University Press	270 13 4	
Audit Fee	5 5 0	
Sundry Expenses	16 10 10	
		292 9 2
Subscriptions and Entrance Fees, paid in advance		27 2 6
Life-Membership Fund Reserve		150 0 0
Excess of Assets over Liabilities:		
As per Balance Sheet, 31 December 1947	1738 1 10	
Add: Royal Society Grant	150 0 0	
Balance of Income and Expenditure Account for 1948	379 0 4	
	2267 2 2	
Deduct: Transfer to Life-Membership Fund Reserve	118 15 0	
		2148 7 2
		£2617 18 10

Assets	£ s. d.	£ s. d.
Cash:		
Westminster Bank: Current Account	298 9 2	
In hands of Treasurer	150 0 0	
Post Office Savings Bank Account	1229 7 3	
		1677 16 5
500 National Savings Certificates		822 17 5
Stock of *Annals of Applied Biology* at estimated value		117 5 0
		£2617 18 10

H. F. BARNES, *Hon. Treasurer*

I certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to me, and, in my opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association according to such books, vouchers and documents.

(Signed) J. B. BENNETT, *Chartered Accountant* } *Auditor*

HARPENDEN, 11 *April* 1949

OBITUARY NOTICE

Dr GEO. H. PETHYBRIDGE

George Herbert Pethybridge, who died at Bodmin on 23 May 1948, was one of the twenty-six original members of the Association of Applied Biologists when it was inaugurated, under its old name of the Association of Economic Biologists, in 1904. There was no precedent for such an Association in Britain at that time, and its first members were indeed pioneers, whose work was to pilot the establishment of most of the applied biological services in this country as we know them to-day. Among all that little band of original enthusiasts, and those who later joined them, there can have been no one who did more, by precept and example, to further the aims of the Association in the field of plant pathology than Dr G. H. Pethybridge.

By the example of the very thoroughness with which he trained himself for a new kind of work in the world; by the honesty and painstaking care in investigation, which he always observed himself and demanded of others; by his classical contributions to our knowledge of potato diseases in particular; by his insistence upon the importance of field studies; and, above all, by the immense amount of critical work that he did to help others in the preparation of their publications, and his staunch friendship to younger workers while they were still unknown, 'Pethy' or 'G.H.P.' as he was affectionately called, won a place in the history of his science and in the memory of his fellows, that is above all awarded honours and public fame.

Born in Bodmin in 1871, Pethybridge came of an old Cornish family; his father was manager of the East Cornish Bank. He was brought up in the Cornish pride of making a 'proper job' of anything to which he turned his hands. His school days were spent in Bodmin, and in Launceston at Dunheved College. How much he learned from school and how much from wandering about the Cornish moors and having to catch his own grass-hoppers when he went fishing for trout in the river Fowey, no one can say. He was a seventh child, and when it was found that he wanted to follow a scientific calling, rather than work in the Bank, he was lucky enough to have no silver spoon put in his mouth. He studied at Aberystwyth, because it was then the least expensive of the University Colleges, and took his Honours Degree in botany and geology at London University. He then earned his living for some years as a science teacher at Kingswood School, near Bath, and at Aske's Hatcham School, near London, until in 1897, after saving every penny, he was able to go to Germany, where he studied under Professors Berthold, Peter, Wallach, Riecke and Fleischmann at the University of Göttingen. His *Arbeit* was an investigation on the effects of inorganic salts on the development of plants, and he obtained his Doctorate in 1899, at the age of twenty-eight.

In 1900 he took up his first post, as a kind of botanical maid-of-all-work, in the Department of Agriculture and Technical Instruction for Ireland, at the Royal College of Science, Dublin. There, as Samuel has already so well said in the *Journal of Horticultural Science*, the young Pethybridge worked in the spirit of adventure and zeal for discovery that characterized botanical research at that time. Original papers on a variety of subjects soon began to appear over his name, including those on leaf spots of *Arum maculatum*, 1903; the vegetation of the district lying south of Dublin, 1905; causes of 'blowing' in tins of condensed milk, 1906; and American gooseberry mildew, 1907. By 1908 he had become Economic Botanist to the Department, and Head of the Seed Testing and Plant Division.

It was in 1909 that the chance for which Pethybridge had been waiting came to him at last. His old chief, J. R. Campbell, asked him to go to the west of Ireland to investigate some mysterious troubles that were being reported on the potatoes out there. Since the disastrous season of 1897 the Department had been promoting potato spraying, and through their efforts

nearly 4000 sprayers had been sold in the west of Ireland alone, but this was not enough; the crops were suffering from diseases other than blight, whose nature was unknown. Campbell insisted that Pethybridge should live with the potatoes, 'sleep in the drills if need be', and study them through their entire growing season. Campbell, who came of peasant stock on the Orkneys, was a man after Pethy's own heart, very unlike some of the 'big-wigs' who thought that he should sit in a laboratory in Dublin, doomed to be forever guessing with a microscope about specimens sent in by others.

Pethybridge seized his great opportunity. He had a corrugated iron hut erected at Clifden near Galway, set it up as a simple field laboratory, and went into lodgings nearby. There, between the months of May and October, each year from 1909 to 1917, he worked undisturbed among the potatoes, sometimes alone, and sometimes with his assistant, Paul Murphy, who was later also to achieve a world-wide reputation. The results of Pethybridge's investigations in Galway, published as yearly reports in the Journal of his Department, and in the Proceedings of the Royal Irish Academy and the Royal Dublin Society, provided a very Hesiod of potato diseases. Stalk-break, blackleg, dry rot, blight, verticillium wilt, degeneration, premature yellowing, common scab and powdery scab—all came under critical review. Many practical conclusions were reached, which were of direct importance to growers, and the light thrown on the simultaneous occurrence of several diseases and disorders—on the complexity of the pathological scene in the field—did much to help the breakaway from the museum-jar mycology of the time, and to show the true part of mycological diagnosis in practical plant pathology. All this work in the west of Ireland, which included the discovery of the causal agent of pink rot and of the occurrence of amphigynous antheridia in species of *Phytophthora*, ranked in historic importance with the greatest of investigations on potato diseases, and was commemorated in 1921 by the award of the Boyle Medal of the Royal Dublin Society.

During his twenty years in Ireland, Pethybridge did not work only on the bacterial and fungus diseases of potatoes. He built up the general work of the Seed Testing Station, took part in the great international wrangle over *Blattrollkrankheit*, which was resolved by the Dutch discovery of leaf roll as a virus disease, and started several important investigations which have since been followed up and greatly extended by others, such as his work on celery leaf spot as a seed-borne disease (1915) and his investigations with H. A. Lafferty into flax diseases (1917 to 1919) at a field station near Coleraine.

In 1923, at the age of fifty-two, Pethybridge resigned his post in Ireland and was persuaded to succeed A. D. Cotton as Mycologist at the recently established Plant Pathology Laboratory of the Ministry of Agriculture and Fisheries at Harpenden. In accepting this post at the call of duty, Pethybridge denied himself the investigatory work in the field which was the light of his life, and devoted all his experience and energy to presiding over the conferences of the fourteen newly appointed Advisory Mycologists for England and Wales, helping with technical advice on the legislative side of plant disease control, and developing the Ministry's plant pathological intelligence service, with an assistant whose capacity for hard work was the very equal of his own, W. C. Moore. The keenness of the young advisers, let loose to explore the occurrence of plant diseases in England and Wales for the first time in history, the comprehensiveness of their reports, and the many fine Bulletins on investigations carried out in those early days of the Advisory Service—all bore witness to the inspiration and unfailing critical help of G. H. Pethybridge. The majority liked him from the first, and shared his sense of humour. A few took exception, now and then, to his brisk way of speaking his mind, especially about work offered for publication too soon, or about what he called 'kindergarten occupations'. Some, who had not done enough writing to know better, were disposed to call him a schoolmaster, because he tried to improve the English as well as the scientific contents of their papers. But all admired his wealth of knowledge and the integrity of his judgements.

His chief failing, perhaps, was a certain lack of sympathy with the childishness that often accompanies scientific talent. He never married, and the lack of children of his own may have deprived him of a gentle understanding of scientific jealousies and illusions of self-importance.

He could never amuse himself by flattering the self-elected great. For them he had a favourite story of two Cornish roadmen, leaning on their spades to watch the approach of a certain Mr Ellis, a rate-collector who gave himself airs. Said the one to the other: 'Mister Ellis...do 'ee know Mister Ellis?' 'Hm!' was the reply, '*Mister* Ellis...'*ee* should 'a been stepped on when 'ee were an egg.' This kind of annihilatory remark, which endeared Pethy to his friends, certainly did not please everybody.

He disliked administrators who sought to make scientists sign time-books, and who wanted to treat them as interchangeable cogs in a piece of State machinery. He cared more for the findings of the humblest field worker than for the illumnati of the great universities. He detested club-life, after-dinner speaking, and most of the activities of politicians, and yet he was one of the best ambassadors for British plant pathology that history is ever likely to ignore. Throughout his working life, and almost up to the day of his death, he was in constant personal correspondence with leading botanists and plant pathologists in many parts of the world. He was a member of many Societies, including the Royal Irish Academy, the Royal Dublin Society, the Linnean Society, the Royal Horticultural Society, and the British Mycological Society, of which he was President in 1926. He raised the standard of our plant pathological services and won respect for them abroad. He made our Advisory Leaflets among the best in the world. He smoothed away many a legislative difficulty because he knew the man at the other end. He knew where research was needed and helped to find workers capable of doing it. He persuaded manufacturers to recruit biologists, and told them how to get their products properly tested before putting them on the market, thereby rendering an invaluable but unseen service to British trade. He kept himself in the background, and never wrote a book, but he gave his knowledge freely for the betterment of many books written by others. At least three of the really indispensable books that are now among the daily working tools of our profession: Brooks's *Plant Diseases*, Wormald's *Diseases of Fruit and Hops*, and Moore's *Diseases of Bulbs*, all owe something to the close scrutiny they received from Dr G. H. Pethybridge before publication.

On his retirement from the Plant Pathology Laboratory at Harpenden in 1936, Pethybridge was honoured by the award of an O.B.E., and promptly forgot it, though he did once say, in a moment of confidence, that he was proud that King George, whom he greatly admired, had given him 'such a nice handshake'. That handshake was well-deserved, but it was not valedictory. In fact, for Pethybridge, retirement meant only a chance to continue working, but with a greater freedom. For over eleven years after that he maintained his scientific correspondence, kept in close touch with his friends, and especially his successors, Geoffrey Samuel and W. C. Moore. He cheered and stimulated all who went to see him, and assisted in the editing of the *Journal of Pomology and Horticultural Science*. He read, corrected, and reshaped innumerable papers, for this and other journals, and always managed to return them within a very few days accompanied by wads of comments in true pethybridgean script and style, which are now greatly treasured by their fortunate recipients.

To know Pethybridge during his retirement, when he had returned to Cornwall, and was living in his sisters' house at Bodmin, was to know a happy man. The little room that he had as a study was crowded with his favourite botanical books, galley sheets draped the chairs, his field boots stood drying by the wall, and piles of correspondence jostled for space on his table with jewel-like mint beetles feeding under lamp glasses. These beetles (*Chrysomela menthastri*) happened to have colonized the neat patch of mint in his garden, and he studied their habits from generation to generation in the utmost detail, partly for the reason that they belonged to the same family as the Colorado beetle. He was then over seventy, his ginger hair paling a little, but still the hardest of workers and the gamest of companions. He forayed the brooks and coppices about his home for fungi and rare Cornish plants, and incidentally discovered *Phytophthora infestans* growing on *Lycium*. Dr Katharine Johnstone was one of his frequent visitors, and he took a great interest in the strawberries of the Tamar valley. To go out botanizing with him was a rare delight, for at the touch of his walking stick every herb of the field would spring into a new prominence—he had a name and an anecdote for every one. At

war-time potato spraying demonstrations, which he often attended, it was good to see the farmers gathered around him, listening entranced to his expert causerie on potato diseases, mingled, as it was sure to be, with reminiscences of Cornish families for miles around—families that he had known as a boy. That was to see Advisory work at its intimate best—and it was something more. It was to see our old warrior of plant pathology, home from his wars, speaking himself to the farmers for whom his whole life work was done, repaying the debts of his childhood, before his ashes were scattered, and his last proof was read. E. C. LARGE